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Controlling Cell Behavior through the Design of Biomaterial Surfaces: A Focus on Surface Modification Techniques

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Surface interaction at the biomaterial–cell interface is essential for a variety of cellular functions, such as adhesion, proliferation, and differentiation. Nevertheless, changes in the biointerface enable to trigger specific cell signaling and result in different cellular responses. In order to manufacture biomaterials with higher functionality, biomaterials containing immobilized bioactive ligands have been widely introduced and employed for tissue engineering and regenerative medicine applications. Moreover, a number of physical and chemical strategies have been used to improve the functionality of biomaterials and specifically at the material interface. Here, the interactions between materials and cells at the interface levels are described. Then, the importance of surface properties in cell function is discussed and recent methods for surface modifications are systematically highlighted. Additionally, the impact of bulk material properties on the cellular responses is briefly reviewed.

1. Introduction

Various tissues or organs in the human body undergo degeneration or loss due to trauma, disease, and aging.[1] The improved understanding of natural tissue healing processes and function has encouraged the scientific community to search for new regenerative approaches such as tissue engineering, which provides potential solutions for regenerating the damaged tissues.[2] Tissue engineering approaches generally include the preparation of specific constructs with specific surface and bulk properties, seeding cells onto the constructs, in vitro tissue maturation, and implanting the cell–scaffold constructs into the site of interest to reconstitute lost tissue.[3,4] As a key component in tissue engineering, scaffolds should be capable of providing a mechanical support and delivering transplanted cells into the desired site.[5] It is expected that the implanted scaffold will evoke the extracellular matrix (ECM) production and gradually get replaced by host tissue.[6] Design and manufacturing of scaffold with ideal characteristics is an important aspect in the current tissue engineering strategies. Specific properties of the scaffold surface lead to increased affinity of biomolecules to the construct.[7] Functionalization approaches can be classified into two main groups: 1) bulk functionalization, such as fabrication of copolymers including blocks from synthetic and natural polymers; and 2) surface functionalization, i.e.,

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manipulating the surface properties to improve cell and material interface.[8] Since cell–scaffold interactions mainly occur at the interface,[7,9] specific properties of the biomaterial such as surface hydrophilicity, free energy, roughness, softness, chemical composition, and morphology strongly affect the transplantation success of constructs.[10] In order to improve these interactions between cells/tissue and scaffold, the physical, chemical, and biological characteristics of material should be optimized.[11] One strategy for modulating the characteristics of material is surface modification, which is considered as a promising alternative for improving cell–material interaction and developing biocompatibility without altering the bulk material properties.[12] A number of techniques for improving the surface properties have been established; however, no comprehensive strategy is available to be utilized for any kind of material. The variations in the surface modification techniques depend on the application and the type of material used. For example, for cardiovascular devices, materials should be blood compatible and antithrombogenic.[13] At the cellular levels, surface modifications should be able to reduce the immunogenicity, decrease the nonspecific adhesion of proteins, and improve the loading of active agents or drugs onto the constructs.[8,14] Likewise, surface modifications contribute to the improved biocompatibility, tailoring degradation rate, enhanced permeability (such as biomolecules' diffusion), electrophysiological stability, contractility, and mechanical properties.[15]

Overall, a comprehensive understanding of various techniques for conjugation and loading of biomolecules onto the surface of scaffold is required for future scaffold design. The conjugation of various functionalized groups and biomolecules onto the material surface contributes to their better functionality and further development for potential applications in tissue engineering and regenerative medicine (TERM).

2. Surface Modification Methods

Surface modification is defined as coating or modifying the surface of a material using physical, biological, and chemical techniques and develops materials with preferred functionality different from the original material.[16] To allow the linkage of adhesive biomolecules to the scaffold, surface should be first activated. The choice of an appropriate technique for conjugation of biomolecules or biomaterials onto the surface of a scaffold is a key issue in TERM. There is a close association between the binding efficiency and the surface properties of scaffolds such as the presence of reactive functional moieties, hydrophobicity, and hydrophilicity.[17] In an attempt to achieve high binding efficiency, a number of modification methods and cell adhesion molecules have been employed.[16,18] Among modification methods, chemical and physical methods have attracted much attention in recent years.[19] The biological modification techniques are the most extensively exploited methods owing to their strong effects on cell adhesion and growth. In this review, we highlight various surface modification methods and then describe different types of cell adhesion molecules that can be exploited to modify surface characteristics of scaffolds.

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2.1. Chemical Modification Techniques

2.1.1. Chemical Adsorption

Chemical techniques can be employed to immobilize biomolecules or bio-/nanomaterials onto the surface of substrates through chemical grafting of functional moieties (silanization, fluorination, sulfonate incorporation, and acetylation) or functionalization of existing functional moieties (reduction and oxidation) (**Figure 1**).[20,21] Chemical adsorption of biomolecules onto the surface of substrates occurs via covalent bond formation.[22] Genipin is an excellent natural cross-linker for chitosan and other biomolecules, and has been used to improve surface topography of electrospun chitosan nanofibers for the design and manufacture of nerve guidance channels.[23] Indeed, an

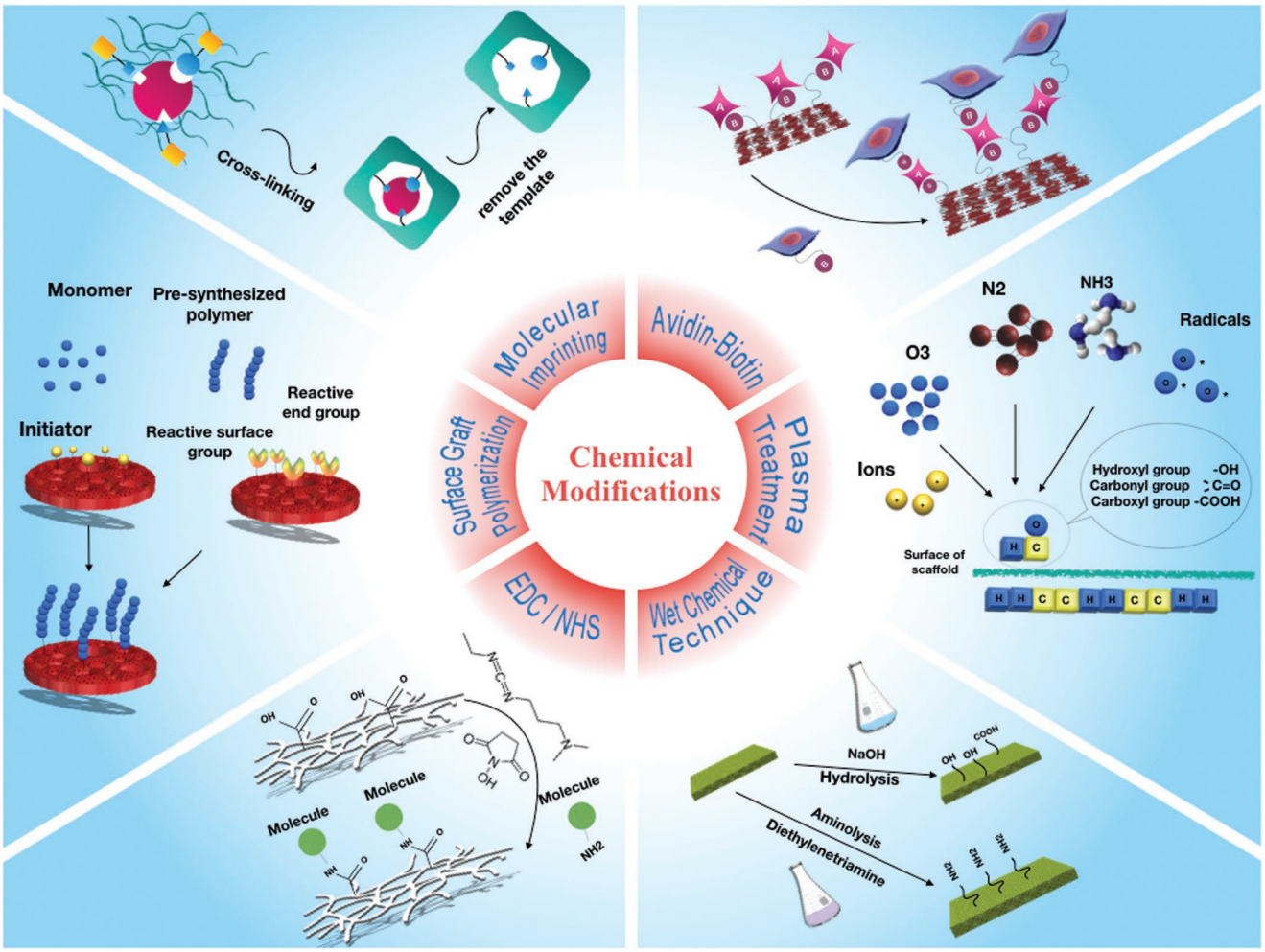

Figure 1. Schematic representation of different chemical surface modification techniques to improve the cell–material interactions.

amine group in the chitosan endures nucleophilic attack at the C—OH group of genipin, leading to covalent bond formation between the aldehyde group and the secondary amine and a double bond formation at the carbon in the *ortho*-position.[24] Previous studies showed resistance to lysosomal degradation and swelling, and a 100% increase of neurite growth rate on the surface of genipin-treated nanofibers.[25] There are several covalent modes to provide chemically reactive surfaces for immobilization of biomolecules to the scaffolds.[26] Covalent modes have advantages including the high controllability for immobilization of biomolecules onto the surface and prolonged availability of biomolecules for promotion of cellular functions.[27] Among them, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide/*N*-hydroxysuccinimide (EDC/NHS), a bifunctional or mediator linker, has attracted much attention from scientific community due to the water solubility of compounds that are produced as waste at the end of reaction.[28] Water solubility of waste products allows their easy washing at the end of the process. Moreover, EDC/NHS linker does not induce any cytotoxicity in the biological systems as it does not participate in the linkage.[28] The choice of carboxylic (—COOH) group–containing surface is an important step for employing EDC/NHS as intermediary linker for covalent immobilization of biomolecules.[29] Covalent immobilization is based on "surface activation" reaction between an amino ($-NH_2$) group on the biomolecules and a succinimidyl ester (—COOSuc)-terminated surface in the presence of EDC/NHS linker.[30] As a promising example of this covalent mode, Guler et al. prepared arginylglycylaspartic acid (RGD)-functionalized polycaprolactone/poly(*m*-anthranilic acid) (PCL/P3ANA) electrospun nanofibers using EDC/NHS as intermediary linker to promote cell adhesion, proliferation, and osteogenic activity (**Figure 2**).[20] P3ANA provides —COOH groups onto the surface for covalent immobilization of RGD peptide. RGD peptide was covalently bonded to nanofibers via activation of —COOH groups on aniline backbone of P3ANA by EDC/NHS linker.[20] Beiki et al. described synthesis of a 3D spongy substrate using human Wharton's jelly to promote wound healing.[31] The authors mentioned that NHS/EDC linker contributed to covalent attachment of —COOH groups from sulfated glycosaminoglycan (GAG) with NH_2 groups of collagen in sulfated GAG–collagen matrices and provided considerable resistance of tissue scaffolds to enzymatic degradation.[31] In another study, Manchineella et al. showed that covalent modifications of silk fibroin (SF) films led to the increased proliferation of human multipotent mesenchymal stromal cells (hMSCs) relative to physical modification of SF

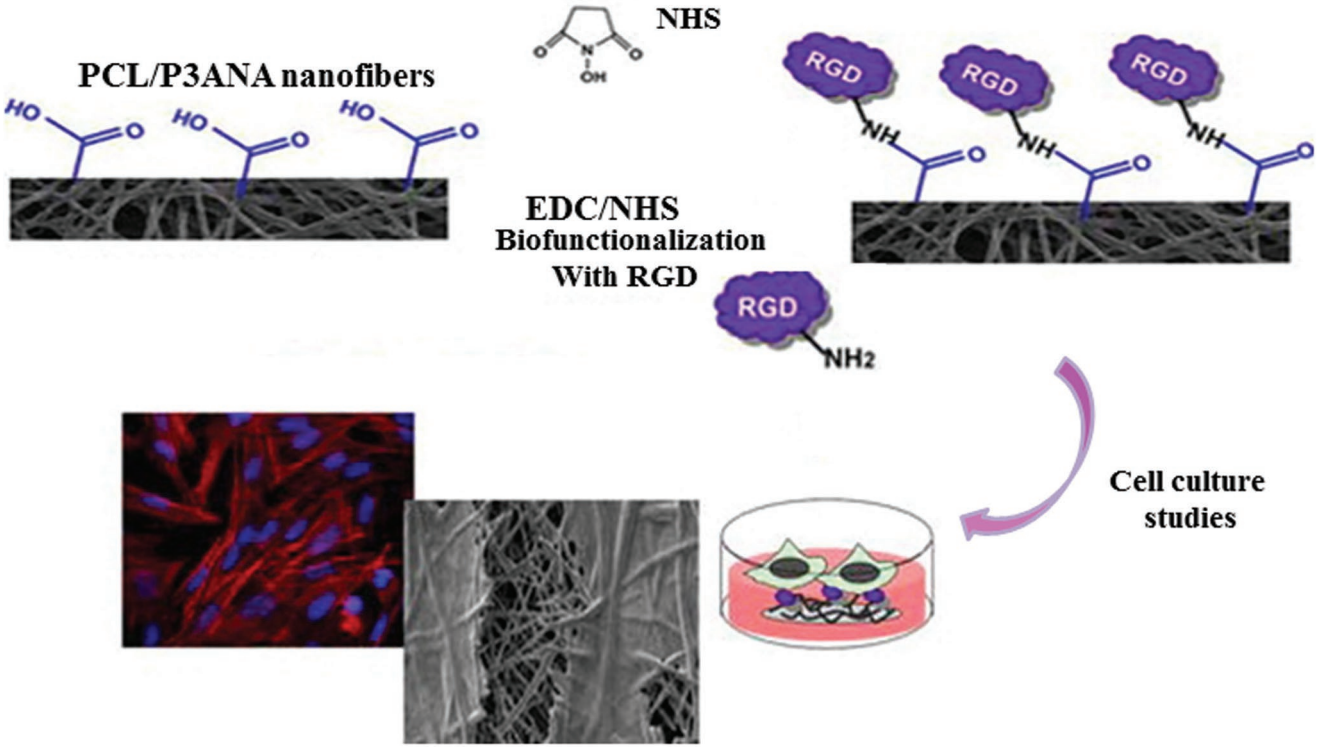

Figure 2. RGD peptide immobilization technique for surface functionalization of biopolymers. Covalent immobilization of RGD onto the surface of PCL/P3ANA nanofibers via EDC/NHS chemistry. Reproduced with permission.[20] Copyright 2016, Taylor & Francis.

films.[32] These findings suggest that covalent modification would be appropriate approach for the long-term culture experiments.[32] Sadeghi et al. demonstrated that EDC/NHS linkers provide sufficient stability of surface active agent, which, in turn, results in increased bioactivity of surface in electrospun poly(lactic-*co*-glycolic acid) (PLGA) scaffold for skin regeneration.[33] Another study by Chen et al. developed platelet-rich plasma (PRP)-functionalized electrospun fibrous and polyhydroxybutyrate (PHB) membranes that were cross-linked using EDC/NHS.[34] In order to introduce hydroxyl (−OH) and aldehyde groups for decoration of dopamine onto the surface, the electrospun PHB scaffolds were treated with hydrogen peroxide (H_2O_2) and glutaraldehyde (GLA) solutions, respectively. The results showed that dopamine molecules act like anchors and covalently immobilize chitosan onto the surface of PHB fibers. Finally, using the EDC/NHS linker molecules, PRP was covalently bonded at the free terminal amines of the chitosan.[34] One of the most interesting linkers that provided precise and sufficient stability of surface active agents onto the surface of biomaterials for tissue engineering applications is maleimide.[35] The maleimide reactive group has been known for its selective reactivity to cysteine residues in protein.[36] In biological sciences, the thiol–maleimide reaction (pH = 6.5–7.5) is used for conjugation and immobilization of the thiol-containing biomolecules such as fluorescent dyes.[37] The maleimide group has been widely used for immobilization of biomolecules on various metallic and glass surfaces.[38] Park et al. fabricated novel poly(ethylene glycol) (PEG)-based hydrogels with different concentrations of thiol-reactive maleimide functional groups via photopolymerization at room temperature.[36] Then, they biotinylated the PEG hydrogels through the nucleophilic thiolene conjugation method to facilitate immobilization of the fluorescein isothiocyanate (FITC)–streptavidin biomolecule on the surface of PEG hydrogels. They reported that the immobilization of FITC–streptavidin in hydrogel can be achieved by changing the density of maleimide groups. They also concluded that the PEG-based hydrogel containing thiol-reactive maleimide functional groups can be used as a platform for cell adhesion and proliferation.[36] Mobasseri et al. introduced −COOH groups on the surface of the nanofibrous PCL scaffolds using the EDC/NHS solution.[39] After removal of unbound EDC/NHS molecules, activated surface was treated with maleimide–PEG–amine solution. Finally, the linker-conjugated scaffolds were incubated with the R-peptide solution overnight. The authors demonstrated that PEG linker decreased steric hindrance and made peptide accessible for cells and resulted in early formation of filopodia from seeded MSCs.[39] Another chemical strategy that can be used to immobilize biomolecules on the surface of scaffolds is the application of adapter molecules.[40] One promising adapter molecule that is widely used for surface modification of scaffolds is avidin–biotin.[41,42] High specificity, affinity, and stability of this binding system affect the mechanical and hydrophilic features of scaffolds and make them suitable substrates for cell growth and proliferation by conjugating biomolecules onto the surface of scaffolds.[43] This binding system is the strongest noncovalent biological interaction and can be unaffected by environmental stimuli such as temperature, pH, most denaturing agents, and organic solvents.[44] Avidin and streptavidin possess four binding sites and create a strong specific and stable binding

with biotin molecules with an affinity constant near 10^{15} M^{-1}.[45] Avidin can be electrostatically attached on the surface of some substrates such as carboxymethyl cellulose (CMC).[46] A common protocol for using avidin–biotin chemistry in the tissue engineering is conjugation of biotin to the cell membrane and immobilization of avidin onto the biomaterial surfaces.[47] Kim et al. described functionalized porous scaffolds based on the avidin–biotin binding system to promote cell adhesion and bone differentiation.[48] Biotinylation of cells was performed using the EZ-Link-Sulfo-NHS-LC-Biotin reagent and the surface of scaffolds was decorated by simple adsorption of avidin or avidin–biotin binding. They found a uniform and rapid adhesion and proliferation of MG-63 cells on the scaffolds compared with the control group and attributed it to the avidin–biotin binding system.[48] On the other hand, the avidin–biotin binding system may act as an effective method to facilitate immobilization of viral vectors to the scaffolds.[49] Currently, much attention has been devoted to the substrate-mediated gene delivery based on a virus–biotin–avidin–biotin–material arrangement owing to multiple merits of concentrated virus vectors in implant sites, improvement of transduction efficiency, and controlled and sustained release of viral particles.[49] However, the avidin–biotin binding system has some drawbacks, including 1) being difficult to create coated surfaces with high coverage densities owing to large size of the (strept)avidin, 2) possibility of cross-linking and clustering effects due to tetravalent affinity for biotin, 3) immunogenicity of proteins in living systems, and 4) limitation of chemical conjugation due to the presence of multiple reactive moieties on the surface of proteins.[50]

2.1.2. Wet Chemical Techniques

Wet chemical techniques are advantageous toward better controlling the crystal phase and surface morphology at microscale and nanoscale.[51] On the other hand, these techniques are easy and permit incorporation of biological and organic components to achieve a variety of biological functions.[51] The enrichment of the surface with favorite functional groups including −OH, $-NH_2$, and −COOH seems to be a promising strategy to influence cellular behavior for TERM purposes.[52] The aminolysis technique is commonly used with EDC/NHS chemistry to introduce primary or secondary $-NH_2$ groups onto the surface of biomaterial.[53] Sangsanoh et al. immobilized laminin onto the surface of PHB scaffold using the aminolysis technique and EDC/NHS as a cross-linker agent.[54] First, the PHB scaffolds were aminolyzed in hexamethylenediamine/isopropyl alcohol solution. After washing with deionized water and drying in vacuum, the aminolyzed scaffolds were immersed in EDC/NHS solution, and finally scaffolds were soaked in laminin solution. The covalently bound laminin–PHB scaffolds markedly enhanced cell adhesion and proliferation (**Figure 3**A).[54] Hoseinpour et al. used EDC/NHS chemistry and surface aminolysis to prepare CMC- or sulfated CMC (SCMC)-modified polyethersulfone (PES) membranes.[55] Surface aminolysis of PES membranes was performed via immersion in an aminolysis solution (10.0 wt% diethylenetriamine (DETA) in water). Then, after removing physically adsorbed DETA by ethanol, PES-NH_2 membranes were incubated in a CMC or SCMC solution containing EDC/NHS and morpholineethanesulfonic acid as solvent. By employing this method, the antifouling properties of membranes were improved and resulted in improved protein adsorption and subsequently initiation of immune responses against membranes was reduced.[55] In another study, poly(L-lactide) (PLLA) nanofibrous scaffolds were aminolyzed to immobilize the −OH, $-NH_2$, and −COOH groups onto the surface.[56] Then, aminolyzed scaffolds were immersed in graphene oxide (GO) solution. GO-coated PLLA nanofibrous scaffolds exhibited better hydrophilicity and performance for nerve regeneration.[56] Another wet technique that can be applied to immobilize −COOH groups on the biomaterial surface is the hydrolysis method. Hydrolysis treatment is a simple technique that can be used to increase the surface roughness and hydrophilicity via NaOH treatment.[57] Yuan et al. used this method to functionalize porous PLGA microspheres using poly-L-lysine (PLL) (Figure 3B,C).[58] Briefly, surface hydrolysis of PLGA microspheres was performed through immersion in excess 0.1 M NaOH solution. Then, the hydrolyzed PLGA microspheres (PLGA-H) were soaked in PLL or FITC-labeled PLL (PLL-*g*-FITC) solution overnight. Hydrolysis treatment resulted in the generation of a homogeneous interconnected porous structure owing to dissolution of the thin polymer around the pores. They found that surface modification with PLL promoted initial cell attachment and improved cell–matrix interactions.[58] Pore size and structure created by aminolysis or hydrolysis may have different effects on cell behavior. For example, 3D porous PCL scaffold with a pore size of 200–300 μm following hydrolysis treatment provided a better environment for osteogenic differentiation of hMSCs. However, the same scaffold with a pore size of 300–450 μm and treated with the aminolysis method showed the most favorable conditions for chondrogenic differentiation of hMSCs.[59] Moreover, it was observed that combined treatment of biomaterial surface by wet chemical techniques and adhesive biomolecules could provide stronger cell attachment.[60] Pilipchuk et al. investigated the effects of four different surface treatments of nonpatterned PCL substrates on cell adhesion: 1) amination (PCL-NH_2 surface chemistry), 2) only hydrolysis, 3) only modification with fibronectin, and 4) hydrolysis pretreatment prior to surface modification with fibronectin.[60] They showed that the combined treatment of hydrolysis and fibronectin coating provided the strongest cell adhesion at multiple time points.[60] Similar to the aminolysis technique, hydrolysis can also be used with EDC/NHS chemistry to covalently bond cell adhesion molecules onto the surface of substrates. However, hydrolysis is pH dependent and detrimental surface degradation may occur.[61] Brown et al. prepared nanofibrous PLGA electrospun scaffolds using the wet electrospinning technique.[62] They hydrolyzed the surface of scaffolds by immersing in 0.01 M NaOH solutions. Then, the hydrolyzed scaffolds were incubated with EDC/NHS solution to treat PLGA scaffold surface with ECM components including collagen I and fibronectin and to evaluate long-term in vitro function of primary human hepatocyte on the surface of scaffolds. They found that incorporation of collagen I on the surface of the PLGA nanofiber scaffolds resulted in higher hepatocyte-specific gene expression, albumin secretion,

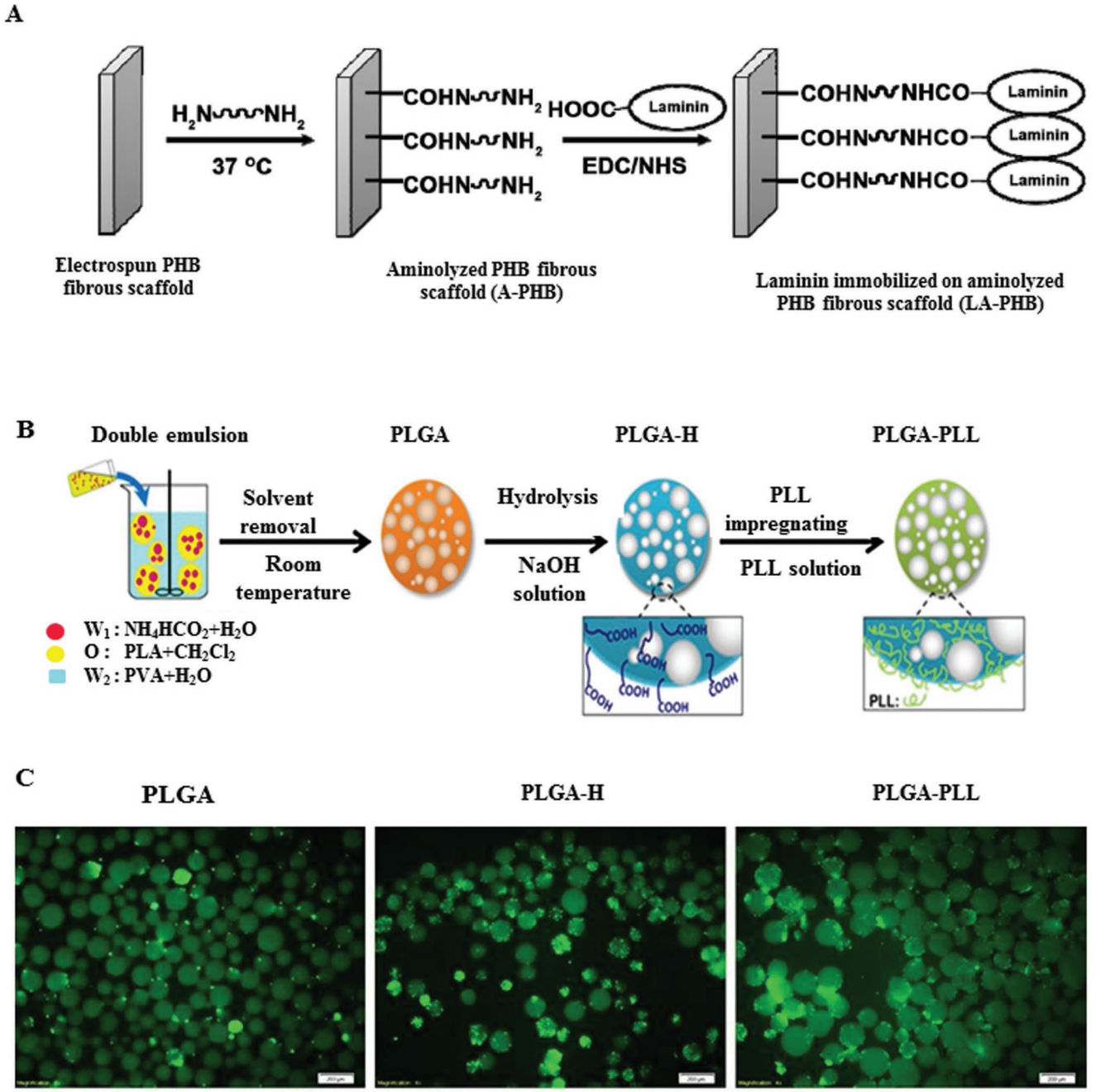

Figure 3. Aminolysis and hydrolysis methods for the immobilization of biomolecules on the surface of biomedical scaffolds. A) Chemical pathway for aminolysis surface treatment using EDC/NHS chemistry to immobilize laminin on the surface of electrospun PHB fibrous scaffolds. Reproduced with permission.[54] Copyright 2017, Springer Nature. B) Surface hydrolysis using sodium hydroxide (NaOH) solution for introducing carboxyl (—COOH) groups on the surface of porous PLGA scaffolds (PLGA-H) and then fabrication of PLL-modified PLGA (PLGA–PLL) microspheres via interaction between cationic and anionic sites of PLL and PLGA-H, respectively. C) Fluorescence images of the PLGA, PLGA-H, and PLGA–PLL microspheres after culturing of MG-63 cells for 10 h. The scale bars represent 200 µm. B,C) Reproduced with permission.[58] Copyright 2018, Elsevier.

and cytochrome P450 catalytic function in comparison with the scaffolds coupled to fibronectin and unmodified PLGA scaffolds. They concluded that cell-laden collagen-bonded PLGA nanofibrous scaffolds provided a specific microenvironment for long-term in vitro survival and function of primary hepatocyte, which can be attributed to the presence of collagen I on the surface of nanofiber scaffolds.[62]

2.1.3. Surface Graft Polymerization

Hydrophobic surface and poor solubility of synthetic polymers are the major drawbacks for their utility as scaffolds for TERM applications.[42] Surface graft polymerization is an efficient approach to modify surface of materials by introducing multifunctional groups and provide long-term chemical

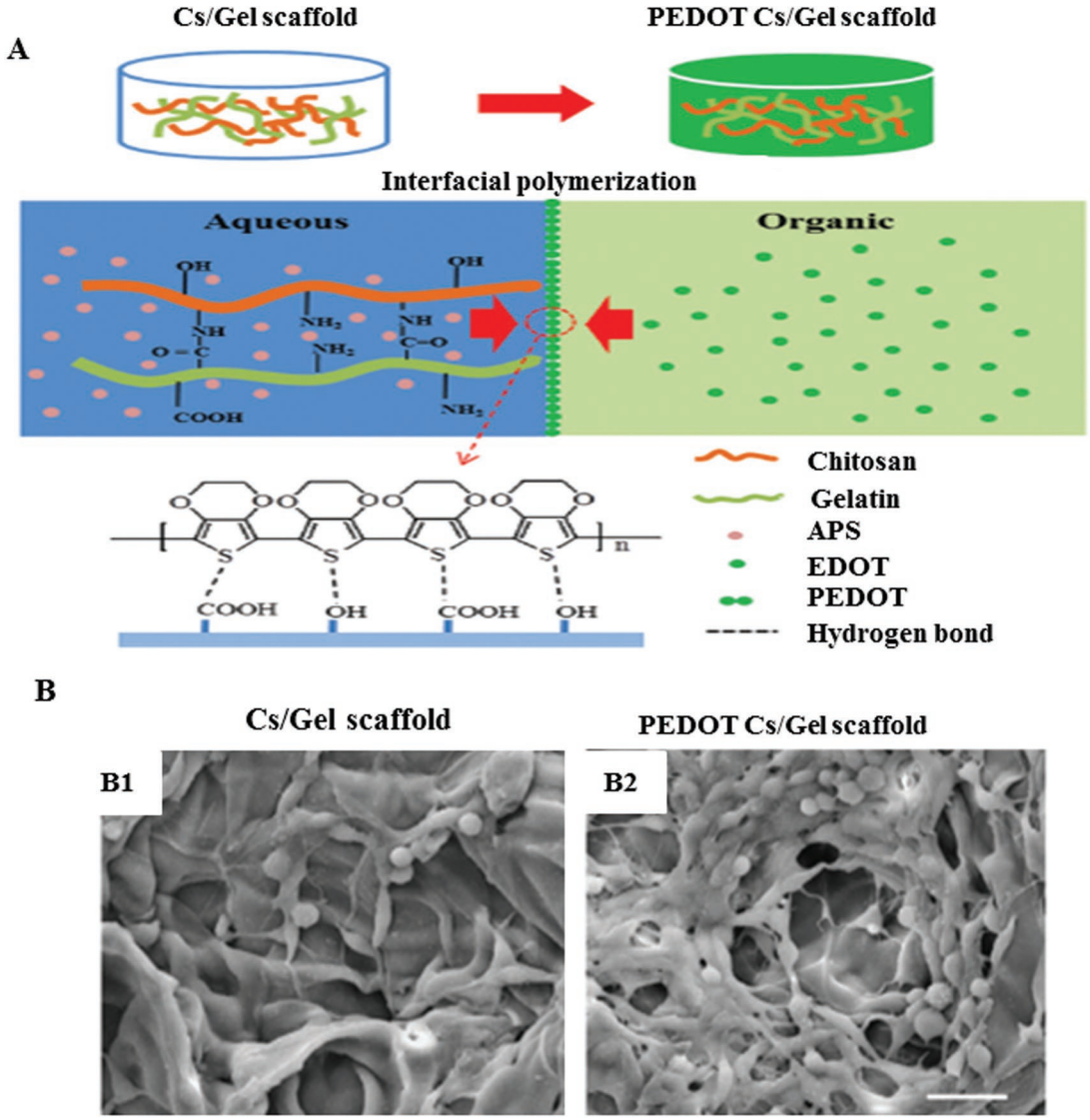

Figure 4. In situ interfacial polymerization technique for the deposition of NPs on the conductive polymer for regenerative medicine purposes. A) Assembling of conductive PEDOT polymer through in situ interfacial polymerization method on the surface of porous chitosan/gelatin (Cs/Gel) hydrogels as a template for neural regeneration. The polymerization of EDOT monomers in the interface of aqueous and organic phases results in the formation of electrically conductive PEDOT-assembled Cs/Gel constructs. B) Scanning electron microscopy (SEM) images of PC12 cells on the surface of B1) Cs/Gel and B2) PEDOT/Cs/Gel substrates after 3 days of culture. Scale bar is 50 µm. Reproduced with permission.[65] Copyright 2017, Royal Society of Chemistry.

stability owing to possibility of covalent immobilization of bioactive molecules on the surface of scaffolds.[63] This method offers numerous advantages, including simplicity, improvement of surface hydrophilicity, low cost, and controllability of surface chemistry without changing the bulk material properties.[64] Wang et al. prepared chitosan/gelatin porous scaffolds assembled with conductive poly(3,4-ethylenedioxythiophene) nanoparticles (PEDOT-NPs) by the static polymerization of PEDOT monomers at the interface between the organic and aqueous phases.[65] In brief, chitosan/gelatin hydrogels were submerged in an aqueous solution of ammonium persulfate that acted as an oxidant. Then, the swelling scaffolds were soaked in cyclohexane solution (EDOT monomer). During the interfacial polymerization process, PEDOT-NPs were formed in situ via forming hydrogen bonds and decoration of PEDOT onto the surface of substrate and resulted in enhanced hydrophilicity, thermal stability, electrical conductivity, and mechanical properties that promoted adhesion and differentiation of PC12 cells (**Figure 4**A,B).[65] Some of bioactive molecules can be deposited on the various hydrophobic or hydrophilic surfaces of polymers through self-polymerization. The previous studies reported that polydopamine (PDA) can be easily immobilized onto the surface of biomaterial through self-polymerization by the oxidation of dopamine in a weak alkaline buffer solution.[66] Overall, in situ surface polymerization technique is an efficient strategy to increase mineralization via chemical interactions between bioactive molecules and polymers during the polymerization process.[67] Dhand et al.

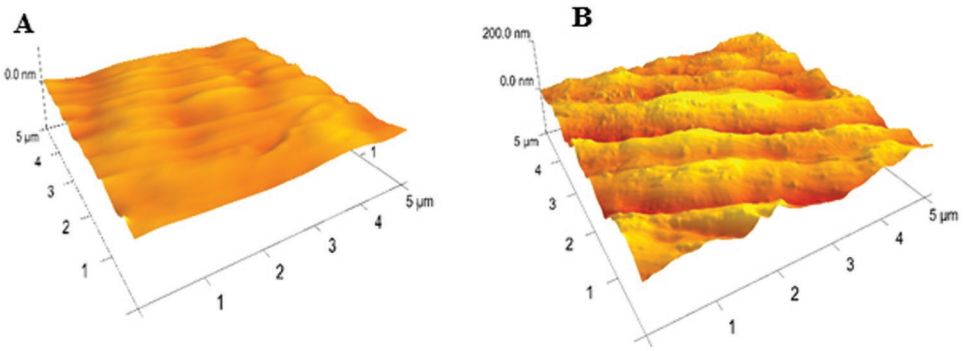

Figure 5. Plasma surface functionalization of PEEK scaffolds for the improved bone formation in the bone implants. Atomic force microscopy images of A) untreated PEEK scaffold and B) the PEEK scaffold after treatment with ammonia/plasma immersion ion implantation. The formation of ripple-like topography at the surface of PEEK scaffold was noticed after plasma treatment. Reproduced with permission.[80] Copyright 2016, American Chemical Society.

exploited this technique to increase surface mineralization of substrate using $CaCl_2$ and collagen mats doped with catecholamines such as dopamine and norepinephrine.[68] They observed that oxidative polymerization of catecholamines in the presence of divalent cations during electrospinning resulted in fabrication of collagen network with soldered junctions. They also found that exposure to ammonium carbonate increased oxidative polymerization of catecholamines and precipitation of $CaCO_3$.[68]

2.1.4. Surface Modification Using Ionized Gases (Plasma Treatment)

Plasma as the fourth state of matter is a mixture of oppositely charged particles, ions, excited atoms, neutral atoms, electrons, radicals, UV photons, and/or molecules.[69,70] The gas plasma can be generated by microwaves, radiofrequency oscillations, and a hot filament discharge that can energize the electrons in excess of the ionization threshold.[71] It has been reported that the gas type and the plasma conditions strongly influence the surface properties of biomaterial through breaking down of the molecular bonds.[72] In general, plasmas are categorized into two major subgroups: thermal plasmas and cold plasmas.[73] Thermal plasmas represent charged or neutral heavy particles and electrons with very high temperatures. Thermal plasmas such as plasma spraying are not usually suitable for the surface modification of thermosensitive materials such as polymers, due to destruction of the surface fine structure like pores during plasma treatment.[74] Cold plasma is characterized by charged and neutral particles and atomic species with low temperature.[75] Cold plasma is a mild and time-saving technique that is extensively used for TERM purposes and contributes to modifying the surface chemistry without destroying the surface structures.[76] Cold plasma treatment is a promising strategy that introduces nitrogen- and oxygen-containing functionalities onto the surface of polymers using NH_3, O_2, air, and N_2 gases.[77] Plasma treatment has been widely applied as a preliminary step to other modification methods such as radiofrequency plasma followed by vinyltrimethoxysilane grafting.[78] The plasma treatment approach allows for tailoring the surface adhesion and wetting features via alterations of surface chemical composition using ionized gases. The advantage of this technique is that the surface properties can be improved without changing the physicomechanical properties of the bulk material. On the other hand, owing to the non-line-of-sight nature, plasma treatment provides the possibility of easy conformal treatment of substrates with a complex geometry.[79] Zhao et al. found that plasma treatment strongly improved osseointegration at the bone–implant interface of polyether ether ketone (PEEK), an organic thermoplastic polymer with mechanical properties similar to human bones, by increasing the surface roughness and hydrophilicity without alteration in the inherent elastic modulus (**Figure 5**A,B).[80] Bolbasov et al. used the plasma treatment technique to modify the surface of bioresorbable electrospun PLLA scaffolds via reactive magnetron sputtering of a metal target (99.99% titanium) under nitrogen atmosphere.[81] They implanted the plasma-treated scaffolds in the subcutaneous area and showed no changes in the physicomechanical features and structural integrity of plasma-treated electrospun PLLA scaffolds after 3 months.[81] In addition, modified scaffolds exhibited an increased surface hydrophilicity with no cytotoxicity as confirmed in vivo.[81,82] It was shown that pure argon and helium plasma discharges result in generation of free radicals. Such free radicals provide possibility of grafting or cross-linking of oxygen-containing reactive groups via their exposure to O_2 or air.[83] More recently, Moriguchi et al. examined the impact of low-pressure plasma on the interconnected porous calcium hydroxyapatite (IP-CHA) scaffold.[84] They found a higher hydrophilicity and osteoconductivity in IP-CHA scaffolds when dielectric barrier discharge plasma was applied in conjugation with oxygen.[84] They used X-ray photoelectron spectroscopy and microfocus computed tomography to characterize the chemical composition and water penetration of inner porous structures after plasma treatment, respectively. The results demonstrated that increasing the levels of oxygen rather than nitrogen onto the surface of scaffold leads to the enhancement of water penetration into the inner porous structures and subsequently improvement of the hydrophilicity and the osteoconductivity of the scaffold.[84] In another study, Surucu et al. demonstrated the effects of different feed gas mixtures on the surface properties of electrospun PCL/chitosan/PCL hybrid scaffolds. They found that employing Ar + O_2 + N_2 gas mixtures markedly improved nanotopography, hydrophilicity, and oxygen functionality of the material surfaces.[85] It should be noted that plasma modification has some drawbacks such as destruction of the surface

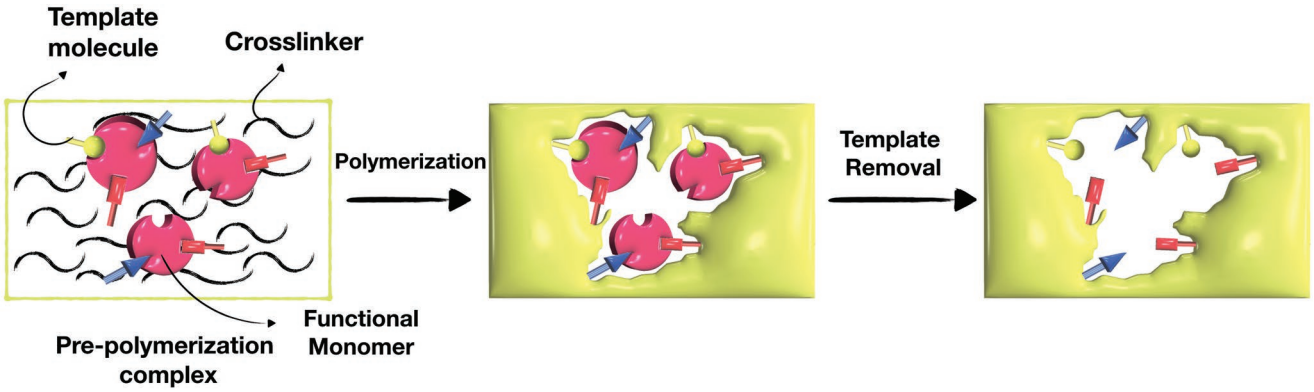

Figure 6. Schematic illustration of the molecular imprinting technology. The molecular imprinting process involves the preparation of suitable template molecule, cross-linker(s), and functional monomer(s) and then the components are mixed in a suitable solvent. The process is followed by the prepolymerization and polymerization steps, and finally the template, cross-linker molecules, and unreacted monomers are washed out.

and production of the toxic compounds onto the surface of substrate following prolonged treatment.[86]

2.1.5. Molecular Imprinting Technology

Molecular imprinting technology is a smart approach to modify material surface by introducing recognition sites for specific molecules.[87,88] Molecular imprinting technology has provided great opportunities in health-related research areas such as immunoassays, chemical sensing, and antibody mimicking.[89] This technology works on natural molecules such as enzyme catalysis or antibody/antigen interactions.[90] In TERM, intelligent matrices should have the capacity to recognize and attach to the ECM proteins and influence cellular behavior. A molecular imprinting technology generally contains three steps: 1) selection of suitable template molecule, cross-linker(s), and functional monomer(s) and prepolymerizing by mixing them in a proper solvent; 2) polymerizing and stabilizing the binding sites and specific cavities; and 3) removing the template and omitting unreacted monomer and cross-linkers (**Figure 6**).[88] Indeed, in this technology, chemical modification of monomer residues in the cavity leads to incorporation of new features like photoresponsivity, on/off switching of binding activity, and fluorescence signaling.[91] To assure the stability of the recognition sites, biostable and biodegradable polymers are used to fabricate scaffolds by molecular imprinting.[92] The great molecular dimensions and chain flexibility of entire proteins as template molecules might decrease capacity and selectivity of molecular recognition. A possible solution is to use short peptide sequences that can be attached to the entire protein.[93] In a promising example of this technology, Yang et al. described the design of nanoimprinted grooves by thermal nanoimprint lithography onto the surface of scaffold to transplant induced pluripotent stem cell–derived retinal ganglion cells to the retina.[94] They used this technology to provide topographical cues by a metallic stamper and showed improved growth of organized axons along the scaffold grooves.[94] Rosellini et al. designed a molecular imprinted polymer with recognition properties toward the fibronectin molecules. The microgel particles were obtained by precipitation polymerization of polymer. They found that particles were able to detect and bind to the fibronectin and promote cell attachment and proliferation.[92]

2.2. Physical Modification Techniques

2.2.1. Simple Physical Adsorption

Safety, cost effectiveness, and biocompatibility are the main advantages of physical methods rather than chemical methods.[95] Physical adsorption has been known as one of the simplest techniques to modify the surface of biomaterials via immersion of substrates in solutions containing the adhesive molecules (**Figure 7**).[96] Growth factors are commonly bound to the surface of the tissue engineered scaffold via noncovalent modes including ionic complexation and electrostatic interactions. Although interactions between surface groups and scaffolds are mainly governed via electrostatic force, other forces such as hydrophobic, van der Waals, and hydrogen bonds are also involved in the physical adsorption.[97] Depending on the protein–surface interactions, the release kinetics of growth factors from the surface of substrates are different and can be affected by the surface roughness, charge, and biodegradation rate of substrate and surface energy.[98] Physical adsorption is commonly employed in combination with other strategies such as covalent bonding.[21,99] Castellanos et al. used different types of surface modification techniques including surface hydrolysis, physical adsorption, and covalent bonding to immobilize adhesive peptides such as RGD, Arg–Glu–Asp–Val), and Tyr–Ile–Gly–Ser–Arg onto the surface of chromium carbide (CoCr) (**Figure 8**).[21] First, surface activation was carried out by basic etching with NaOH solution. To immobilize adhesive peptides through covalent bonding, activated surface was silanized by soaking of CoCr substrates in *N,N*-diisopropylethylamine solution and 3-chloropropyltriethoxysilane in anhydrous toluene under nitrogen atmosphere. They showed that both physical adsorption and covalent bonding could immobilize the cell-adhesive peptides onto the surface of substrates, and a higher amount of cell-adhesive peptides were observed after the silanization process.[21] A simple physical adsorption method is limited as the cell adhesion molecules might be detached from the surface and results in adverse repercussions on cell

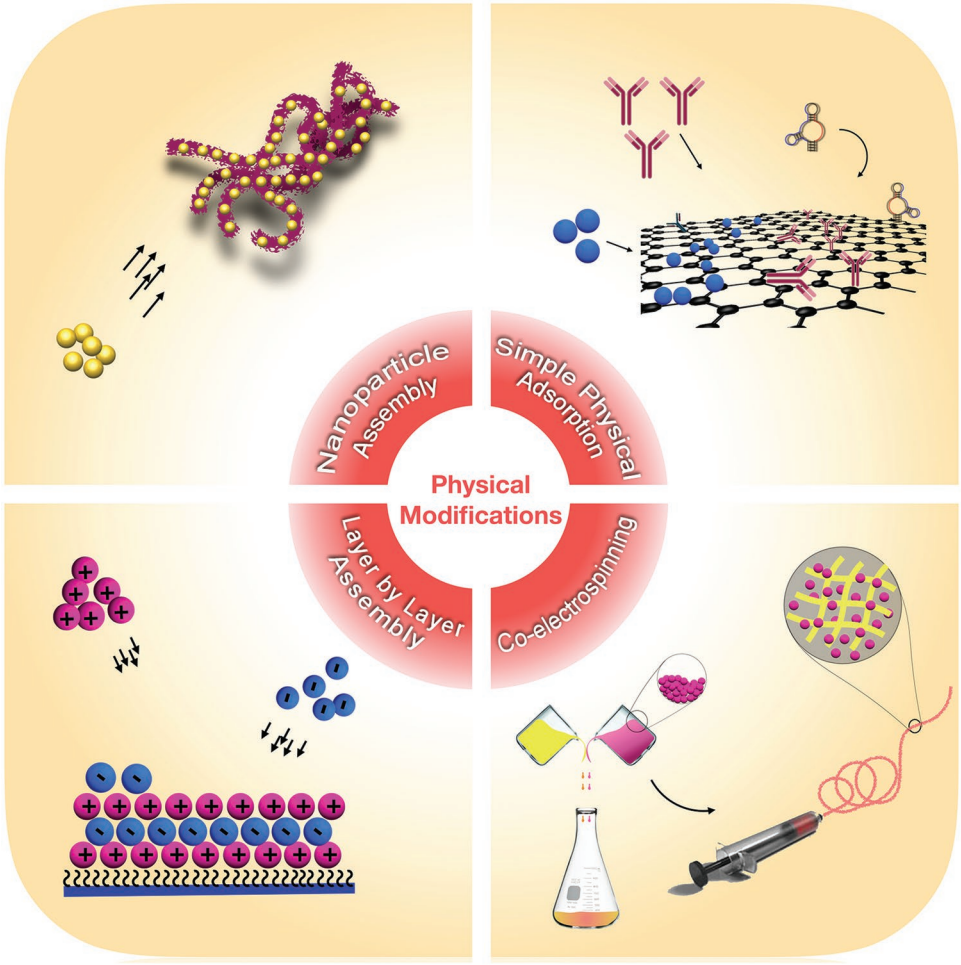

Figure 7. A summary of different physical techniques for surface modifications of biomaterials.

adhesion, growth, and differentiation.[100] Moreover, lack of controllability on the orientation of adsorbed cell-adhesive biomolecules is another drawback that highly decreases the efficiency of this method.[101] Chen et al. investigated the effects of physical adsorption and covalent attachment of laminin molecules on the surface of electrospun silica nanofibrous scaffolds (SNF2).[102] The physical coating was performed by immersing in laminin solution. The chemical bonding was carried out by soaking in an ethanol solution with 3 M (3-aminopropyl)triethoxysilane (APTS), then incubation of the APTS-modified SNF2 in a cross-linker solution, sulfo-(succinimidyl-4-[*N*-maleimidomethyl]cyclohexane-1-carboxylate) (SMCC), and finally immersion of the APTS–SMCC-modified SNF2 in laminin solution. They confirmed that covalent attachment of laminin leads to constant neuritis extension and prolonged biochemical stimulation of PC12 cells, while the physical coating of laminin onto the surface of substrates failed to provide the same effect (**Figure 9**A,B).[102]

2.2.2. Layer-by-Layer Assembly

Layer-by-layer (LBL) multilayer assembly is able to provide surface coating on precisely controlled scales (from a few nanometers to several micrometers).[103] In the LBL technique, oppositely charged polymers (polyanions and polycations) are deposited on the charged substrates via an electrostatic force.[104] Serial exposure of an inherently charged substrate to solutions containing oppositely charged species results in their electrostatic deposition and formation of ultrathin and uniform films.[105] In addition to electrostatic force, hydrophobic, covalent, and hydrogen-bonding interactions are other forces that can be involved in assembling multilayer thin films through the LBL technique.[105,106] Precise control of the coating properties, capability of low-cost production, creation of a biomolecule-friendly environment with mild conditions, production of homogeneous layers with controlled thickness, the possibility of incorporation and controlled release of growth factors/biomolecules/therapeutic agents, and versatility for modification of all available surfaces are the most important advantages of the LBL multilayer assembly method.[107,108] Since the incorporation of insoluble and uncharged agents into the multilayer assembly is difficult, the LBL technique can act as a reservoir for charged therapeutic agents or growth factors.[109] Zhang et al. employed this approach to provide multilayers comprised of heparin, a negatively charged polysaccharide, and collagen encapsulating nerve growth factor (NGF), a positively charged protein, onto the surface of aligned PLLA nanofibrous scaffolds for a

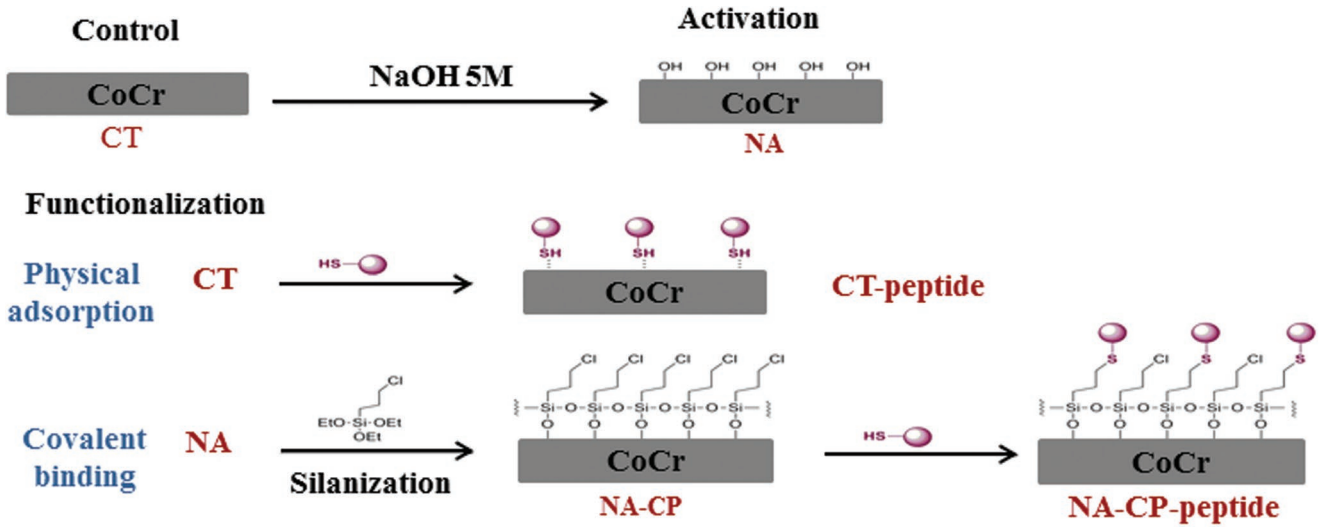

Figure 8. Functionalization of material surfaces with cell-adhesive peptides to improve cell adhesion and proliferation. The surface of CoCr substrate was first activated and then functionalized with simple physical adsorption or covalent bonding through physisorption. *Abbreviations*: CT, the nonactivated substrate (or the control group); NA, the activated substrate; NA-CP, the silanized surface. Reproduced with permission.[21] Copyright 2017, Elsevier.

controlled and tunable release of NGF.[110] Recent studies have taken advantages of this approach to improve cell adhesion, proliferation, and differentiation onto the surface of different substrates.[68,110,111] In regard to LBL assembly, the properties of substrates can be controlled by manipulating processing parameters such as molecular weight, pH, concentration of the adsorption species, functional moieties, ionic strength, nature of polyelectrolytes, and humidity.[112] Indeed, surface chemistry and morphology of material are considered to be a pivotal determinant in their assembly.[113] Biomaterial morphology is essential and the physical interactions in self-assembly are based on material morphology.[114,115] The LBL assembly in combination with the surface grafting technique has the capacity to improve the physical–chemical attributes of the 3D scaffolds due to the assembling of polyelectrolyte multilayers (PEMs) onto the surface of the biomaterials.[114] Nugroho et al. prepared 3D PLLA microsphere scaffolds with combined surface treatment by surface grafting and LBL assembly method.[116] The prepared PLLA microspheres were surface grafted with acrylic acid under UV light and used as primary substrate for building up PEMs. Then, neutral poly(acrylamide) and cationic poly(allylamine hydrochloride) polymeric polyelectrolytes were alternately assembled onto the surface of the poly(acrylic acid)-grafted PLLA microspheres through the hydrogen-bonding and electrostatic interactions, respectively.[116] They found that the combined surface treatments improved the physicochemical features of the 3D microsphere scaffolds.[116] On the other hand, large surface area-to-volume ratio and the inherent hydrophobicity of electrospun synthetic polymeric scaffolds are major disadvantages of these biomaterials that can trigger a sequential chain of pathophysiological cascades, including adsorption of nonspecific proteins, recruitment of macrophage, and finally fibrosis at the tissue–scaffold interface.[117] An ideal technique for surface decoration is expected to eliminate nonspecific protein adsorption and permit host macrophage phenotype modulation.[118] To this end, Qian et al. prepared heparin disaccharide (HD)-modified nanofibrous scaffolds with combined surface treatment by the LBL assembly approach and click chemistry technique to regulate the foreign body reactions to nanofibrous substrates for tissue engineering purposes. The PCL nanofibers were manufactured and functionalized using the LBL self-assembly technique with SF (**Figure 10**).[119] To induce β-sheet formation, PCL nanofibers were treated with methanol and nitrogen gas. Then, treated fibers were chemically modified with HD using EDC/NHS. The authors generally observed that IL-4 molecules were strongly adsorbed onto the surface of HD-modified nanofiber and their sustained release induced macrophage polarization toward M_2 macrophages and resulted in anti-inflammatory responses.[119]

2.2.3. Nanoparticle Assembly

The bioactivity of scaffolds can be tuned by modifying the surface at nanoscale.[120] The successful assembly of NPs on the surface of substrates provides biomimetic scaffolds with specific functionalities for TERM applications.[121] The unique properties of NPs can increase the functionality via improvement in wettability, surface bioactivity, mechanical and opticoelectrical properties, possibility of biomolecules' immobilization through physical adsorption, and increasing the binding sites for cell attachment and proliferation.[121] The large surface area-to-volume ratio of NPs leads to their facile interaction with cells and biological molecules.[122] Unique opticoelectrical properties of gold nanomaterial were employed to improve the conductivity of substrates for nerve tissue regeneration.[123] Additionally, chemical conjugation of functionalized gold NPs (GNPs) onto surface of thiolated nanofibrous scaffolds increased osteogenic differentiation capacity of human adipose tissue–derived multipotent mesenchymal stromal cells (**Figure 11**).[3] The possibility of modifying surface without prefunctionalization and trading off the intrinsic physicochemical properties of substrates via the NP assembly technique sounds promising for clinical applications.[124] Among different NPs, PDA is

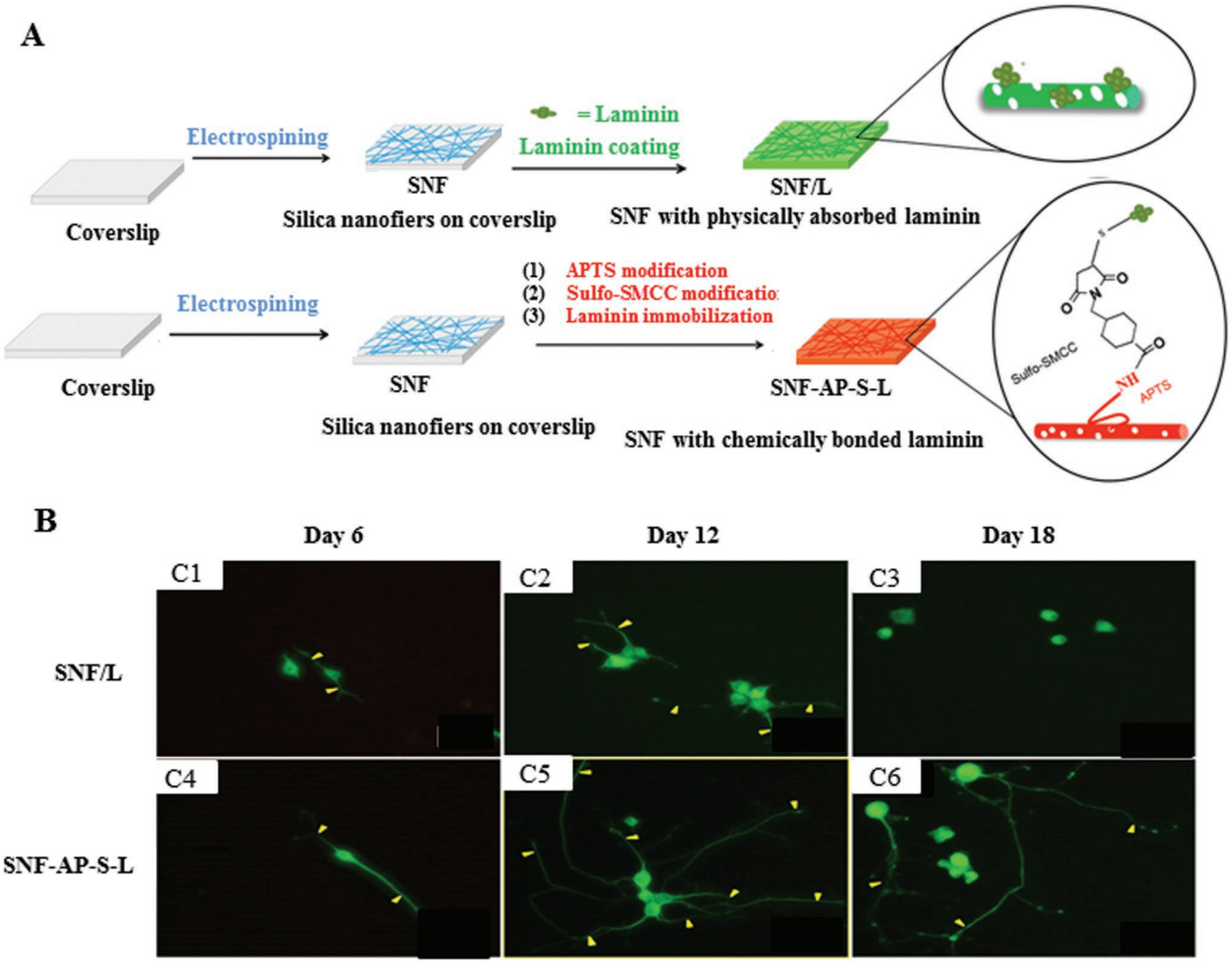

Figure 9. Surface modifications and biofunctionalization of the silica nanofibers. A) Simple physical coating and covalent immobilization of laminin onto the surface of SNF scaffolds. B) The neural differentiation of neuron-like PC12 cells on the surfaces of physically treated laminin-coated SNF scaffolds (SNF/L) and chemically treated laminin-modified SNF (SNF-AP-S-L). Both substrates showed biocompatibility and bioactivity during 18 days of culture. However, PC12 cells seeded on the surface of covalently coated SNF-AP-S-L scaffolds (C4–C6) showed enhanced neurite outgrowth and higher potential to support neural differentiation compared with SNF2/L group (physically adsorbed surface) (C1–C3). Reproduced under the terms of a Creative Commons Attribution 4.0 International License.[102] Copyright 2018, the authors, published by Molecular Diversity Preservation International.

particularly promising due to the presence of multiple reaction sites on the surface of porous scaffolds without prefunctionalization.[125] In an interesting study, Wang et al. functionalized the β-tricalcium phosphate (β-TCP) scaffolds by soaking them in the PDA NP solution.[126] It was observed that the immobilization of PDA NPs onto the surface of β-TCP scaffolds was strongly dependent on the soaking time. The authors mentioned that the use of PDA NPs as coating surface agent can provide anchors for immobilization of biomolecules onto the scaffold surface. They also reported high affinity of modified β-TCP scaffolds to bovine serum albumin, which resulted in improvement of osteoinductivity (**Figure 12**).[126] The NP assembly technique can be employed to overcome the weak mechanical strength of scaffolds that may limit the clinical applications.[127] Graphene is one of the NPs that has attracted tremendous attention by the scientific community owing to its fascinating superior electrical, mechanical, thermal, and optical properties, cost-effective synthesis, capability of bandgap engineering, facile surface decoration, size tailoring, excellent biocompatibility, and degradability.[128] Xie et al. developed a biomimetic mineralized hierarchical GO–chitosan scaffold by assembling the NPs onto the interfacial area of substrate.[129] The electrostatic interactions between the functional carboxyl groups of GO and the amine groups of chitosan provide excellent biocompatibility and mechanical strength. They biomimetically mineralized octacalcuim phosphate onto the surface of GO–chitosan scaffold. Moreover, Ag NPs and bone morphogenetic protein-2 (BMP-2) were immobilized onto the surface to improve antibacterial capacity and osteoinductivity of scaffolds, respectively. The results demonstrated improved MSCs' proliferation and differentiation in vitro and bone healing capacity in vivo.[129] In another study, and to improve the antibacterial activity, mechanical property, and the denaturation temperature, assimilation of synthesized Ag–pectin NPs in a self-assembly

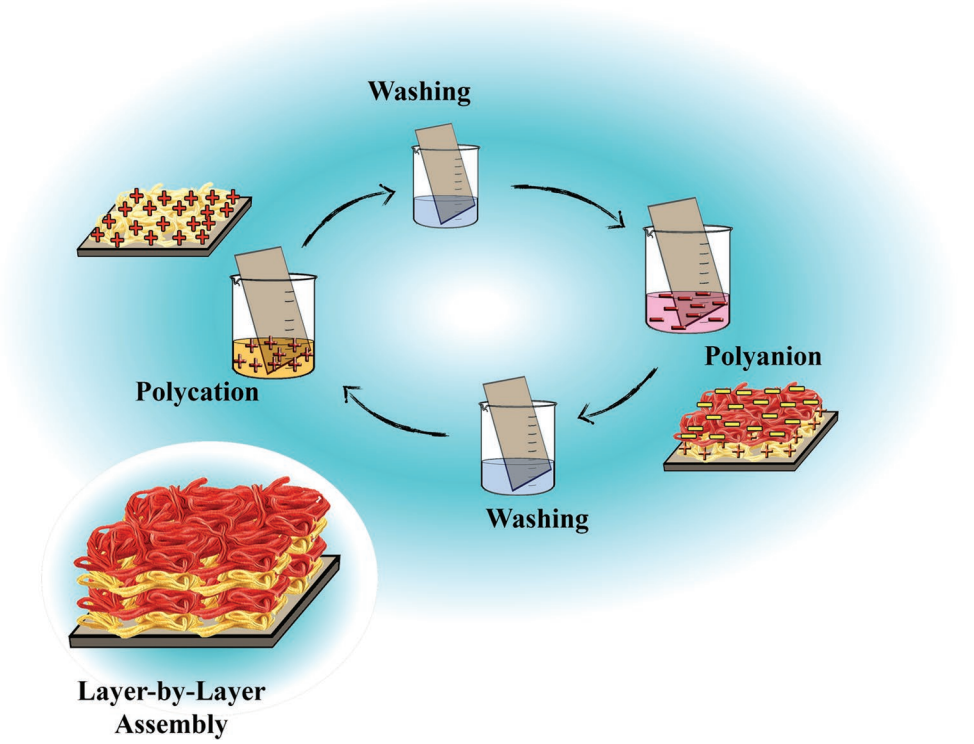

Figure 10. Schematic illustration of the LBL technique for biomaterial surface modifications.

process of collagen was performed.[130] The adsorption of protein onto the surface of scaffolds can be modulated by modifying with NPs and other surface active agents.[131] Based on this concept, Yassin et al. designed a copolymer poly(L-lactide-*co*-ε-caprolactone) scaffold functionalized with nanodiamond particles by a vacuum technique for bone regeneration. The results showed that significant changes in the nanodiamond particle–water solution in the vapor phase in vacuum chamber caused distribution of NPs onto surface of scaffold. They found that substrate surface decoration with nanodiamond particles not only promoted mineralization capability and osteogenic metabolic activities but also hampered protein adsorption and subsequently fibrous capsule formation and progression of chronic inflammation via improvement of the surface hydrophilicity.[132] One of the advantages of using the NP assembly method for modification of scaffolds is that it increases the roughness and hydrophilicity of surface, thereby increasing affinity of adhesive molecules onto the surface of substrates.[133] To achieve this goal, Chen et al. prepared SF-NP-decorated PLLA composite scaffolds through the phase-inversion technique using supercritical carbon dioxide. The SF-NP-decorated PLLA composite showed a high affinity for albumin immobilization.[134] Overall, the surface topography and biochemical cues are of utmost importance and need to be investigated for the design and manufacture of biomaterial scaffolds.

2.2.4. Coelectrospinning

Electrospinning technology has been widely used to manufacture scaffolds for TERM applications.[135] Electrospinning technology has several advantages such as easy handling owing to the simplicity of the process, cost effectiveness, and possibility of utilizing a variety of biomaterials.[136] The successful application of this technology for tissue engineering depends on rendering the fiber surface for promotion of cell attachment.[137] Coelectrospinning of surface active agents such as functional polymer segments and NPs allows achieving this goal.[138] Taskin et al. found that the surface decoration of PCL scaffold with PDA through single-step wet electrospinning created a 3D scaffold with high hydrophilicity and excellent biocompatibility for hMSCs (**Figure 13**).[138] In a recent study, a combined surface treatment by surface grafting and coelectrospinning techniques was introduced.[139] Briefly, TiO_2 NPs were first reacted with dimethylolpropionic acid (DMPA) to introduce −OH groups. Then, hexamethylene diisocyanate and PCL diol were dissolved in a mixture of *N*,*N*-dimethylformamide/toluene to provide the poly(ester–urethane) (PEU) prepolymer with isocyanate groups. The DMPA-functionalized $nTiO_2$ was added to the resulting solution to create PEU-grafted TiO_2 NPs (PEU-*g*-$nTiO_2$). Then, PEU-*g*-$nTiO_2$ was mixed with PEU urea (PEUU) solution and to fabricate the fibrous PEUU scaffolds reinforced with PEU-*g*-$nTiO_2$. The results showed that the surface grafting and coelectrospinning techniques improved the Young's modulus and tensile stress of fibrous PEUU scaffolds for bone tissue engineering applications.[139] One promising strategy toward better mimicking of in vivo microenvironment and controlling cell proliferation is incorporation of ECM molecules in electrospun scaffolds.[140] Bhowmick et al. described the design of biomimetic electrospun scaffolds by coelectrospinning of chondroitin sulfate, sulfated hyaluronan, and gelatin to generate a suitable microenvironment

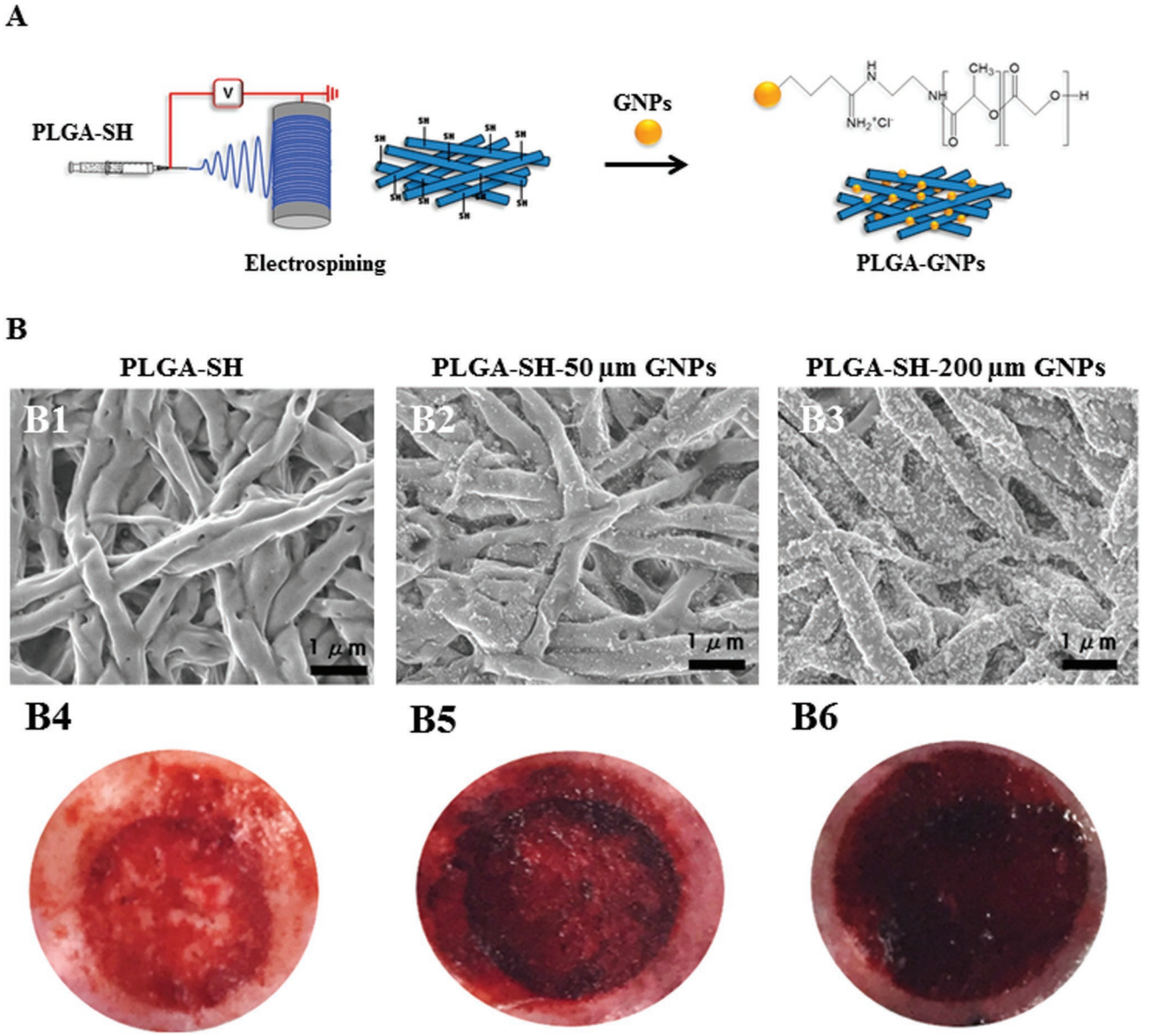

Figure 11. The NP assembly technique for the generation of biocompatible and bioactive polymers for tissue engineering applications. A) Assembly of GNPs onto the surface of thiolated poly(lactide-*co*-glycolide) nanofibrous (PLGA-SH). B) SEM images of B1) the PLGA-SH nanofibers, B2) PLGA-SH with 50×10^{-6} M GNPs (B2), and B3) 200×10^{-6} M GNPs. The osteogenic differentiation of human adipose tissue–derived stem cells (B4–B6) was increased in the experimental groups with higher amount of GNPs (B6). Reproduced with permission.[3] Copyright 2018, Elsevier.

for the attachment of hMSCs, keratinocytes, and fibroblasts (Hs27). Final scaffolds resulted in a remarkable increase in cell number (≈5-fold) compared to the control group (10% gelatin scaffolds).[140]

3. Cell Adhesion Molecules for Surface Modification

The native ECM molecules have attracted tremendous attention to improve the cell-adhesive properties of scaffolds.[141] RGD is an amino acid sequence present in ECM proteins such as vitronectin and fibronectin and selectively binds to the cell transmembrane receptors and subsequently enhances cell adhesion.[142,143] Aside from RGD, there are many integrin-activating domains into structure of ECM proteins that can enhance stem cell attachment or recruitment.[142] For example, Pro–His–Ser–Arg–Asn is an amino acid sequence, found in fibronectin, whose activation results in the enhancement of signaling responses that initiate stem cell recruitment.[144] Despite these merits, ECM proteins have several major shortcomings that limit their clinical use, such as potential risk of pathogen transmission, unsolicited adverse immune response (against cadaveric or xenogenic proteins), and bioactivity loss owing to conformational changes during conjugation to scaffolds.[145]

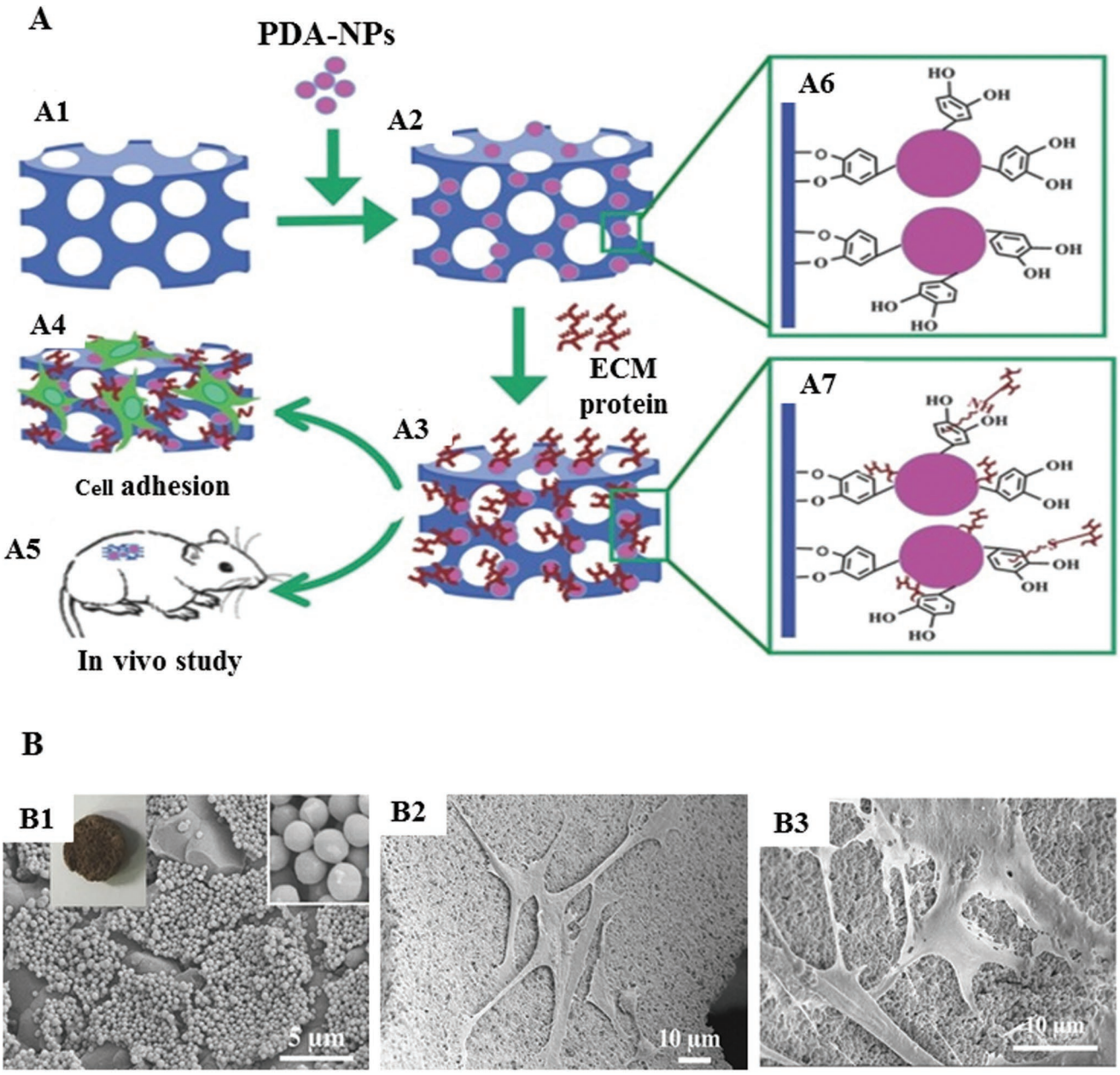

Figure 12. Polydopamine nanoparticle (PDA-NP) assembly represents an efficient strategy for the surface modification of porous scaffolds. A) Immobilization of PDA-NPs on the porous β-TCP scaffolds (A2, A6), adsorption of ECM proteins (A3, A7), cell adhesion (A4), and in vivo implantation of cell-seeded scaffolds (A5) for the purpose of bone regeneration. B) SEM images of scaffolds after 48 h incubation in PDA-NP solution (B1). The bone marrow multipotent mesenchymal stromal cells were seeded on the surface of PDA-NP–β-TCP scaffolds and showed significant adhesion after 1 (B2) and 3 days (B3). Reproduced with permission.[126] Copyright 2016, John Wiley & Sons.

Adhesive peptides could be prepared on large scale and in a short time and have shown great promise for TERM applications without bioactivity loss.[146] Polyornithine, a synthetic amino acid chain with positive charge, was applied as a coating layer on poly(lactic acid) (PLA) and polypyrrole nanofibers and improved the cell adhesion without any toxicity.[147] Antibodies have also been used as surface decorators to improve the functionality.[148] Although both chemical and physical approaches have been utilized for binding of antibodies onto the surface of scaffolds, choosing an appropriate technique often relies on the original features of the substrate.[149] In the case of natural substrates (containing hyaluronic acid and collagen), owing to the inherent presence of reactive moieties, antibodies can be covalently bonded onto the surface.[150] Additionally, lack of inherent reactive moieties on the surface of synthetic substrates confers with direct adhesion of antibodies. The use of an intermediate layer including polymers, charged proteins, and hydrophobins, a class of cysteine-rich peptides, converts unreactive surface of synthetic scaffolds to suitable substrate for antibody anchorage.[151] As a promising example of surface modifications with antibodies,

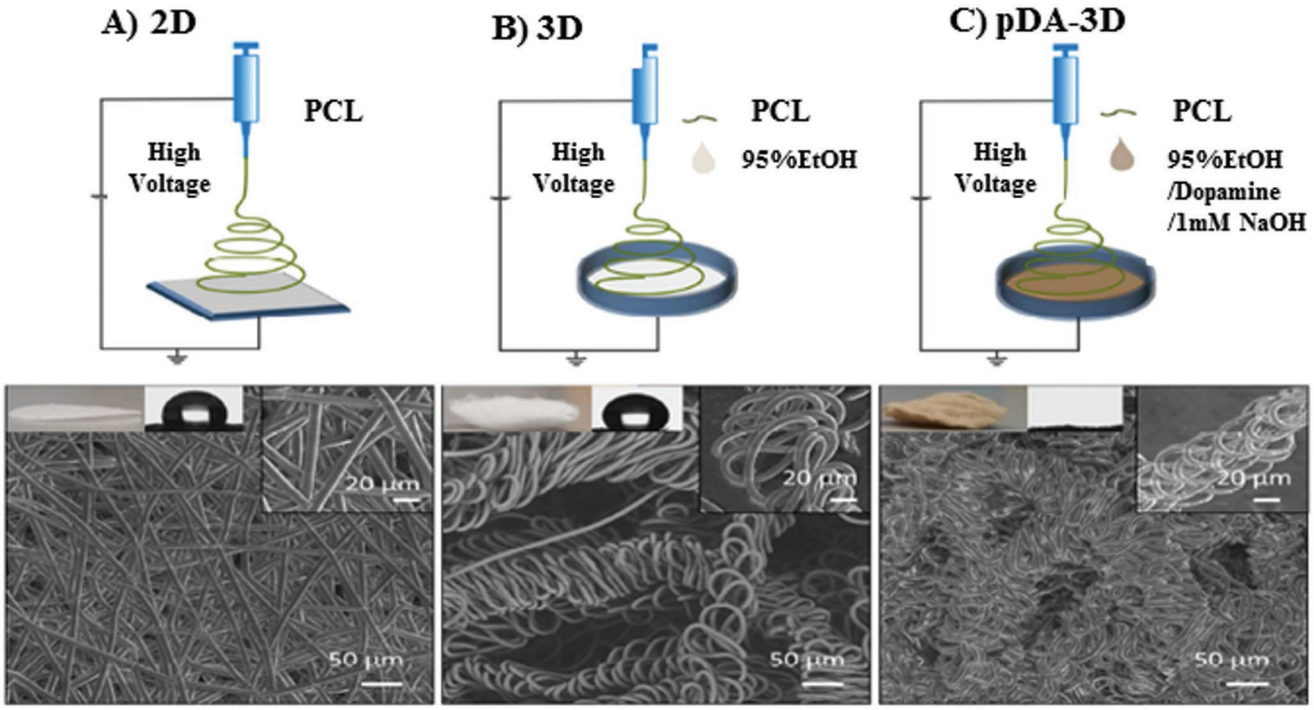

Figure 13. Functionalizing coiled microfibrous scaffolds to enhance cell colonization and differentiation. A–C) Schematic representation and SEM analyses of 2D and 3D electrospinning. A) 2D and B) 3D electrospinning of PCL. C) 3D electrospinning of PCL–polydopamine (PCL-pDA) into ground coagulation bath collector for preparation of the PCL-3D and PCL-pDA-3D coiled microfiber scaffolds using the coelectrospinning method. Reproduced with permission.[138] Copyright 2016, American Chemical Society.

Shi et al. covalently conjugated stem cell antigen-1 (Sca-1, a common surface marker of stem cells) monoclonal antibody on the surface of collagen substrates and showed enhanced recruitment of autologous Sca-1-positive cells and ultimately better cardiac tissue regeneration in mouse model.[152] Likewise, Zhang et al. used hydrophobins as reactive intermediates to improve conjugation of anti-CD31 antibody (key CD marker of endothelial cells) on the surface of PCL scaffolds and observed improved retention and attachment of endothelial cells in immobilized anti-CD31 antibody scaffolds.[149]

Aptamers, synthetic single-stranded oligonucleotides, are another class of biomolecules that can link to the specific target molecules of various types of cells and promote cell attachment.[153] Aptamers as surface decorators have several advantages relative to other classes of ligands such as peptides and antibodies, such as strong affinity to the target cell, inexpensive production, and low immunogenicity.[154] Growth factors, soluble signaling proteins or steroid hormones, are another classes of biomolecules that can be used to modify the surface of substrates and improve cell adhesion, recruitment, proliferation, and differentiation.[80,155] Overall, the design of growth factor releasing substrates can not only influence cellular responses but also increase therapeutic activity and stability of growth factors in coping with chemical enzymatic or chemical degradation.[98] On the other hand, changeability of growth factors in response to biologically relevant stimuli such as light, temperature, pH, and enzymatic degradation makes them appropriate candidates for the design of smart scaffolds.[156]

4. Effects of Bulk Properties on Cellular Behavior

The bulk of a biomaterial indicates physiochemical properties of material that maintain during the lifetime of the implant. Although the interactions at the material interface are a determinative factor for various types of interactions of the implant with the living tissues, the bulk properties of the material are other determinant parameters that should be considered in the design and manufacture of scaffolds (**Figure 14**). Here, the bulk properties of biomaterials are briefly highlighted. Then, we focus on the surface properties of materials and their effect on cellular biological responses.

4.1. Biomaterial Source

Over the last decade, fabricated scaffolds for numerous medical applications are made of natural and/or synthetic material sources with some advantages and drawbacks.[70,157] Several sources of natural materials such as hyaluronic acid, SF, GAGs, fibrinogen, collagen, and chitosan have received much attention from scientific communities as they contain essential components present in the native ECM.[70,158,159] Such biomaterials represent appropriate biocompatibility, biodegradability, and recognition sites for cells to attach.[70] Scaffolds fabricated by natural materials are advantageous in that they possess RGD binding sequences that facilitate interactions with the cell surface receptors and provide a microenvironment to control cell fate, while engineered synthetic scaffolds may require additional

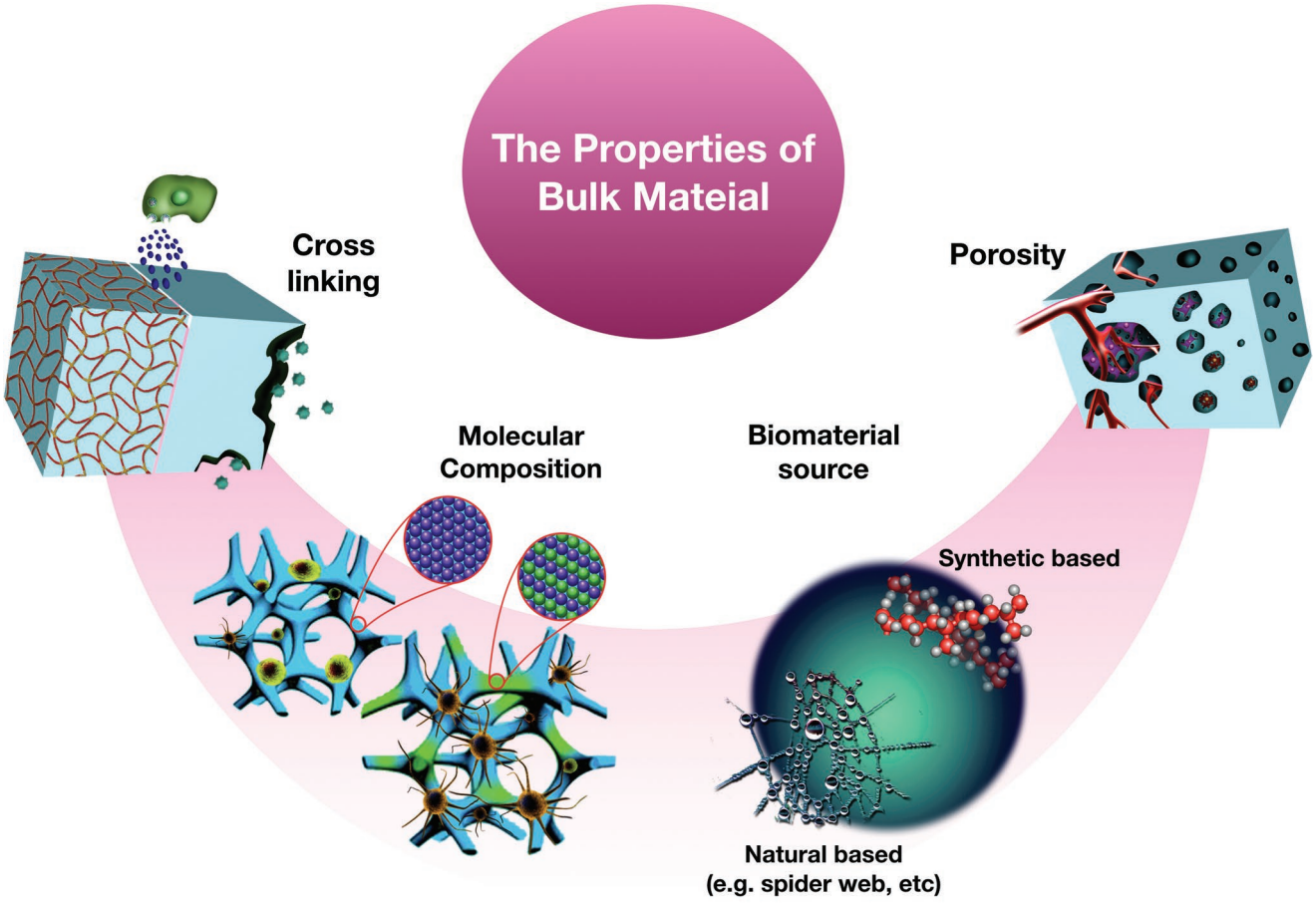

Figure 14. Schematic representation of scaffolds' bulk properties that may affect cellular behaviors.

modification before any applications.[158] Despite the advantages of natural materials, several shortcomings such as high cost of production, difficulty in purification and sterilization, inadequate mechanical properties, and rapid degradation rate often present challenges and limit their translation into the clinical practice.[160] Mimicking the native cell microenvironment by introduction of ECM components into the biomaterial has become a promising strategy to overcome the limitations in regard to cell proliferation and bioactive mediator stabilization.[159] GAG derivatives are important components of ECM. Previous studies demonstrated that sulfate groups of GAGs are able to interact with cell-derived growth factors, thus influencing their bioactivity.[70,161,162] Additionally, GAGs due to the presence of negatively charged, repeating disaccharide units in their backbone and low immunogenicity are promising candidates for TERM practices.[161] van der Smissen et al. examined this concept by coating the surfaces of the macroporous PLGA scaffolds with collagen/GAG mixture.[162] The ECM-coated PLGA scaffolds provided a suitable 3D microenvironment for a homogeneous fibroblast distribution, proliferation, and matrix synthesis throughout the entire scaffold compared with uncoated scaffolds.[162] Another source for preparing the medical implants is the synthetic material group. In order to reinforce the weakness in mechanical features of natural materials, synthetic biomaterials have been used in combination to provide scaffolds with ideal chemical and mechanical properties.[163] On the other hand, hydrophobicity and lack of bioactivity of synthetic materials may result in unfavorable cellular responses and poor cell–matrix interactions.[164] Additionally, the release of toxic products after degradation of these materials limits their clinical applications. For example, during the degradation of PLLA and polyglycolic acid scaffolds, carbon dioxide is produced and therefore the local pH is decreased, which may induce tissue necrosis.[157,165] It has been reported that the surface negative charge of PLGA-NPs may affect their mucoadhesive attribute and bioavailability in drug delivery systems. Therefore, surface modifications can improve surface potential and mucoadhesive properties of PLGA-NPs.[166] In this case, Shi et al. coated chitosan on the surface of tolbutamide-loaded PLGA-NPs (TOL-PLGA-NPs) and switched the zeta potential from negative to positive and improved the bioavailability of drug-loaded NPs. The results indicated that the coating of TOL-PLGA-NPs with chitosan improved the thermostability and therefore NPs could release TOL in a sustained manner at pH 7.4 compared with TOL-PLGA-NPs. In addition, in vivo treatment of the diabetic rat by oral administration of chitosan-coated TOL-PLGA-NPs presented great hypoglycemic effects.[118]

Alternatively, surface modification strategies provide this possibility to compensate drawbacks of the synthetic materials, including the low biocompatibility, bioactivity, and lack of the specific cell attachment sites.

4.2. Molecular Composition

Molecular composition of scaffolds can affect their physiochemical properties such as rigidity/flexibility, hydrophilicity/hydrophobicity, and degradability of scaffolds, which in turn influence specific cell functions.[167] For example, although both PLA and PCL are linear aliphatic polyesters, their different molecular compositions result in higher flexibility, hydrophobicity, and lower degradability of PCL compared to PLA.[168] In addition, polymer composition with high acrylate content provided moderate wettability, increased bioactive molecule absorption, and promoted colony formation of human embryonic stem cells.[169] In our previous study, we also applied a 3D polyvinyl alcohol (PVA)/chitosan/pre-polyurethane scaffold with different concentrations of chitosan as an artificial substitute for meniscus regeneration. We observed that scaffolds with higher amount of chitosan had better cell adhesion and cell proliferation. Additionally, gene expression analysis showed that the scaffolds with higher amount of chitosan had higher expression of cartilage-specific genes.[170] Therefore, employing biomaterials with different compositions for fabrication of blend scaffolds can be a smart approach to design constructs with optimal bulk properties.

4.3. Porosity

Porosity and pore size and morphology play important roles in determining cellular phenotype, cellular behavior, and ECM secretion onto the surface of scaffolds.[171] Macropores (pores >50 μm) are able to determine cell growth and colonization, while micropores (pores <50 μm) influence protein adsorption onto the scaffolds.[172] Moreover, the impact of porosity in neovascularization as a key point in the survival of tissue engineered products after transplantation has been well established.[173] Artel et al. showed that the larger pore size (160–270 μm) resulted in extensive and rapid angiogenesis throughout the porous PEG hydrogels.[174] Ma et al. found that substrates with low porosity (30% porosity and pore size of 0.849 μm) promoted metabolic activities and initial cell proliferation rate, whereas matrices with high porosity (39% porosity and pore size of 0.896 μm) exhibited higher levels of cell aggregation and differentiation.[175] In another study by Di Luca et al., increasing trend in ECM mineralization and osteogenic differentiation onto the surface of scaffolds was observed in scaffolds with larger pore sizes because of increased supply of oxygen and nutrients in the scaffolds.[176] Moreover, Matsiko et al. concluded that scaffolds with larger pore size (300 μm) displayed a stronger cartilage-like matrix deposition, cell proliferation, and chondrogenic gene expression compared to substrates with smaller pore sizes (94 and 130 μm).[177] Although higher porosity can contribute to effective mass transport and subsequently results in more cell ingrowth, larger pores or higher porosity may compromise the mechanical properties of the scaffold.[178] Kim et al. used four types of PLGA scaffolds with pore sizes of 90–180, 180–250, 250–355, and 355–425 μm.[179] They found that although scaffolds with the largest pore size displayed higher cell proliferation and more ECM production, the weaker mechanical properties of scaffolds with larger pore sizes limited their applications.[179] The PLGA scaffolds with pores size of 180–250 μm provide appropriate mechanical strength while supporting cell proliferation and are the most suitable substitutes to treat nucleus pulposus disease.[179]

Constructs with proper porosity and homogeneous interconnected pores have shown promising results for TERM purposes. The porous network in scaffolds can mimic the native ECM architecture allowing cells to interact with their microenvironment. It should be noticed that the pore size and geometry differ in human tissues; therefore, the porosity and pore architecture should be engineered based on target tissues and the anatomical sites.

4.4. Cross-Linking Strategies for Modulating the Scaffold Features

Cross-linking treatments are promising strategies to create scaffolds with excellent physical and chemical properties and control cell behavior through improvement of structural, mechanical, and thermal stability depending on the cross-linking degree.[180] Cross-linking process results in 1) decrease in the viscosity and elasticity (as the degree of cross-linking increases, polymer chains become less elastic and viscous, so they do not stretch much, and also become more rigid and even brittle), 2) insolubility of polymer, and 3) increase in glass transition temperature, mechanical strength, and toughness due to the formation of strong covalent bonds between polymer chains and decrease in flexibility.[181] Based on the kind of polymer, cross-linking can occur via polymerization of monomers, formation of covalent bonds between polymeric chains by introducing different chemicals agents in the presence of heating and/or pressure, physical cross-linking via UV treatment, microwave irradiation, and dehydrothermal treatment, and enzymatic cross-linking using genipin and glycidoxypropyltrimethoxysilane.[181–183] Sun et al. showed that GLA cross-linking agent could improve mechanical properties and increase resistance to biodegradation of collagen sponges.[184] Benameur et al. prepared a scaffold containing chitosan sponges using two purines, adenosine 5′-diphosphate (ADP) and guanosine 5′-diphosphate (GDP) as cross-linker agents, and showed that unlike ADP, GDP molecules were able to form intermolecular hydrogen bonds. This phenomenon resulted in the formation of quadruplex crystals and increased crystallinity of GDP scaffolds.[185] Further studies showed the GDP–chitosan-based scaffolds provided more organized microstructure for activity and proliferation of encapsulated MC-3Tc cells.[185,186] The results demonstrated that different cross-linkers can be used to investigate their impacts on scaffold microstructural integrity, cellular responses, and signaling pathways in the context of chitosan-based scaffolds' applications in TERM. Davidenko et al. studied the effect of reducing the EDC/NHS concentrations on cellular functions of collagen- and gelatin-based substrates.[187] They found that a tenfold decrease in carbodiimide cross-linking maintained the corresponding cell-reactive carboxylate anions available for integrin-mediated cell interactions with collagen substrates. They also claimed that the degree of cross-linking can influence cell behavior through alterations in the porosity and stiffness.[187] The emerging evidence has also shown that

the degree of cross-linking needs to be controlled to retain the number of available essential cell binding motifs onto the surface of the scaffold. It has been reported that cells can sense the slight variation in scaffold properties. For example, higher cross-linking resulted in significant increase in cell spreading and growth through formation of focal adhesion that provides sites for signal transduction from ECM into cell.[188] Additionally, cross-linking can increase water stability and mechanical property of scaffold as well as influence pore size of the scaffold.[183,189]

In conclusion, the cross-linking process markedly improves stability and resistance to degradation of the scaffold as well as changes the hydration properties, surface hydrophilicity, and microstructure of the scaffold.

5. Effects of Surface Properties on Cellular Behaviors

Based on the nature of biomaterial surface and types of bioactive agents and biomolecules that are used for functionalization, the performance of biomaterials in living tissues is changed (**Figure 15**). Surface coating technologies with bioactive agents and biomolecules are commonly used to better mimic the tissue microenvironment.[190] For instance, chemical modifications of bioactive ceramic materials such as strontium-doped calcium polyphosphate with similar mineral components to the bone tissue could decrease their palpable brittleness and improve mechanical properties suitable for bone tissue regeneration.[126,191] Surface chemistry, topography, chemical composition, wettability, stiffness, dimensionality, porosity, and degree of cross-linking are fundamental parameters that must be taken into the account in the design of substitute for medical applications.[192–194] By manipulating surface properties of implants such as surface charge, functional groups, and topography, initial cell attachment can be tuned in a controlled manner.[195,196]

5.1. Surface Chemistry

The influences of chemical functional groups and surface chemistry on the cell–material surfaces have been widely investigated. The association of surface chemistry with wettability and surface charge affects the cell adhesion, cell shape, cell proliferation, and differentiation.[193] Yu et al. investigated the effects of surface chemistry on human dental pulp stem cells (hDPSCs) using a range of self-assembled monolayers (SAMs) with different functionalizing groups such as methyl ($-CH_3$), $-NH_2$, $-COOH$, and $-OH$.[193] They found that hDPSCs attached, proliferated, and displayed osteocyte-like morphology on the surface modified with $-NH_2$ groups compared to other self-assembled monolayers. Surface modifications using $-NH_2$ functional groups may be employed as an efficient strategy to improve osseointegration, osteoconductivity/osteoinductivity, and biocompatibility of dental implants.[193] Additionally, Bai et al. reported that bone marrow MSC (BMSCs) cultured on substrates modified with $-NH_2$ and $-OH$ groups exhibited a fibroblastic morphology while on scaffolds modified with the $-COOH$ and $-CH_3$ groups displayed rounded shapes.[197] Wang et al. developed the SAMs of alkanethiolates on gold surfaces terminated with $-CH_3$, $-OH$, and $-NH_2$ groups and examined the effects of various surface chemistries on behavior of neural stem cells (NSCs).[198] They observed that $-CH_3$ groups induced

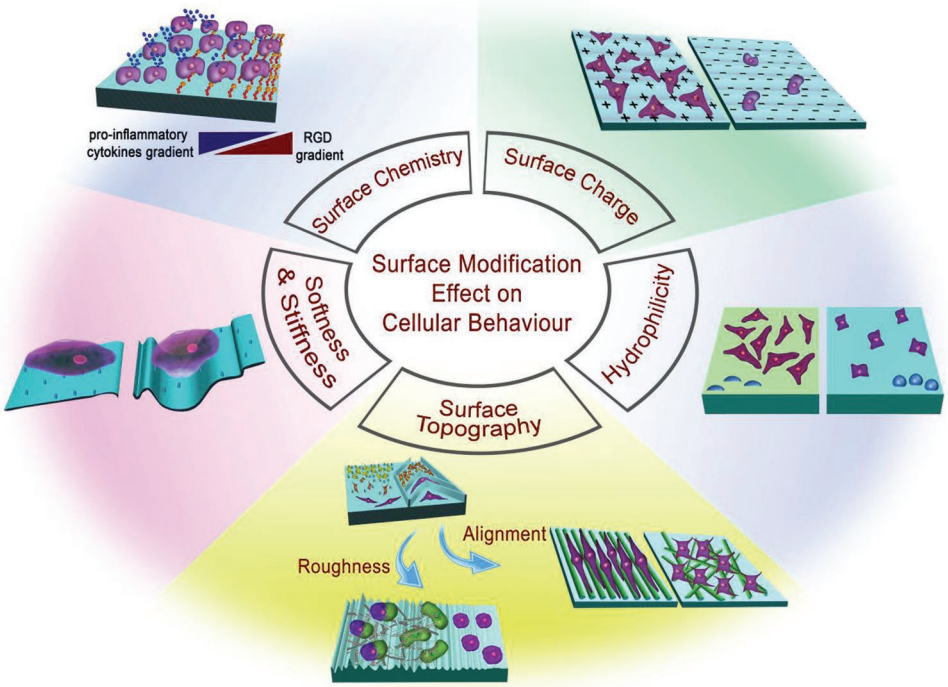

Figure 15. Schematic demonstration of surface properties that may modulate cellular behaviors.

genes involved in cell death, while $-NH_2$ groups induced genes involved in cell attachment and axon growth. In another study, fabrication of titanium implant containing $-OH$ or $-SO_3H$ groups on the surface induced Ca/P deposition.[199]

Surface chemistry strongly affects materials' biocompatibility and immunogenicity. Taken together, the current literature indicates that the manipulation of surface chemistry can be considered as a suitable strategy to manipulate the specific properties of various materials for therapeutic applications.

5.2. Surface Topography

Cells are able to respond to the topographical structure of the underlying surface and modulate their alignment and orientation along the surface.[191] To date, many research studies based on topographical modifications have been performed toward improving the cell–material interface, with the aim to increase tissue healing and improvement in cell anchorage and attachment to the implants.[191,200–203] Current nano- and microfabrication techniques for applying surface topography are self-assembling systems, electron beam lithography or photolithography, particle synthesis, microcontact printing, replica casting or molding, sandblasting, electrospinning, and chemical etching. Previous studies showed that the cellular responses to the surfaces can be classified with respect to the surface patterning scale.[142,201] Micropatterning of surface principally influences cell position, growth, morphology, and cytoskeletal reorganization, whereas nanopatterning of surface can enhance the surface energy and improve the adsorption of protein, cell migration, spreading, proliferation, differentiation, gene expression, and eventually tissue–implant integration.[201]

Unique properties of surface topography patterns such as high stability, cost-effective manufacture, and the easy controllability make them excellent candidates to control cell function and tissue regeneration. Surface roughness and surface pattern as the main components of surface topography will be discussed in the following sections.

5.2.1. Surface Roughness

The most important aspect of surface topography is surface roughness, which indicates the texture of surface of a material and is determined by measuring the surface protrusions or depressions.[204] It was found that rough and smooth surfaces induce different kinds of cell responses; for example, osteoblast cells prefer rougher surface, periodontal fibroblast cells attach better to smoother surfaces, and epithelial cells adhere to the smooth surfaces.[204] Various surface treatment techniques such as plasma treatment,[205] grinding, chemical and mechanical etching,[206] grit blasting,[207] 3D printing,[208] lithography, micromachining, acid etching, ion etching, and electrospinning[94] have been developed to create and improve roughness on the surface of biomaterials. There are two categories of materials tested for studying the impact of roughness on cellular functions. The first category of biomaterials is metallic constructs such as titanium, platinum, and stainless steel, and the second category is polymeric biomaterials.

The roughness on the surface of titanium implants by laser peening significantly increased adhesion and differentiation of osteoblasts.[202] Deligianni et al. produced regular roughness (Ra: 0.320, 0.490, and 0.874 μm) on the surface of Ti-6Al-4V using metallographic paper.[209] The results showed that the roughness significantly influences cell attachment and also a great number of cells were attached to the rougher surface of Ti-6Al-4V (<2 h).

Hallab et al. reported that increase in surface roughness of polymeric materials led to increased attachment of 3T3 fibroblast cells on the surface, but similar result was not observed in metallic biomaterials after increasing the surface roughness.[203] Electrospun substitutes have been assessed as promising scaffolds in tissue engineering since they can mimic the micro- and nanoscale properties of normal ECM. In this regard, Chen et al. investigated the differentiation of hMSCs on the poly(ethylene oxide terephthalate)/poly(butylene terephthalate) electrospun scaffolds with high (Ra = 71 nm) and low surface nanoroughness (Ra = 14.3 nm).[210] They observed that the expression of osteogenic genes, BMP-2, osteopontin, and runt-related transcription factor 2 was increased in scaffolds with higher surface roughness. In addition, the scaffolds with lower surface roughness could induce better cell proliferation and chondrogenic differentiation after 7 days of culture.[210] Faia-Torres et al. showed that PCL scaffolds with roughness of 0.93 μm could induce osteogenic differentiation in hMSCs (**Figure 16**).[211] Human coronary artery cells were tested on the smooth and rough surfaces using PLLA scaffolds. The expression of endothelial genes was increased on the surface of smooth rather than rough scaffolds. Moreover, endothelial cells exhibited a round and cobblestone-like morphology on the rough and smooth surfaces, respectively.[204]

Current studies open new insights into tailoring the surface roughness with the aim to control cell responses for TERM. The effect of different roughness patterns on the cellular responses is discussed in next sections.

5.2.2. Surface Pattern

In order to explain cellular response to the surface pattern, we classify surface patterns into anisotropic and isotropic patterns. Anisotropic surface refers to the direction-dependent and systematically oriented patterns such as grooves and ridges on the surface. The isotropic pattern is a surface with no directional orientation. This surface contains micro-/nanoscale topographical patterns such as pillars, protrusions, pits, and circular, triangular, star, and square shapes.[9,191]

Incorporation of nanotopographical features at the surface of the biomaterial such as metallic glass led to mitigation of high levels of macrophage fusion on substrates that, in turn, result in reduction of the foreign body response.[212] Ngandu Mpoyi et al. studied the effect of nanotopography on the adsorption of proteins and cell behaviors through design of a nanostructured polycarbonate surface forming 150 nm diameter and ≈90 nm deep pits and found a strong adsorption of fibronectin within the interpit spaces and inside the nanopits.[213] In addition, the authors explored that mimicking the pit patterns resulted in greater cell attachment and

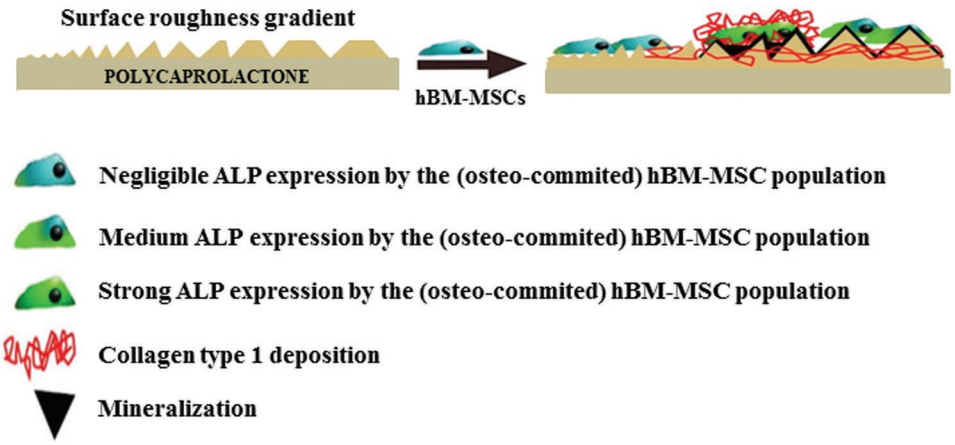

Figure 16. A surface roughness gradient research showed that the surface roughness exerts profound effects on the cell morphology and cell fate. The impact of engineered surface roughness gradients of PCL on the osteogenic differentiation and the expression of alkaline phosphatase protein by human multipotent mesenchymal stromal cells. Reproduced with permission.[211] Copyright 2015, Elsevier.

myogenic differentiation of immortalized mouse myoblast cell line, C2C12, compared to a flat surface.[213] Hierarchical surfaces, containing micro- and nanostructured patterns, have been shown to have the strongest influence on the cell functions.[68,214] In a recent study, Xu et al. reported that the hierarchical micro-/nanostructures and nanosized bioglass in the nanofiber scaffolds have higher bioactivity and accelerate tissue regeneration.[215] Khampieng et al. selected PCL films with various surface topologies to investigate protein absorption and behavior of mouse-calvaria-derived pre-osteoblastic cells. They showed that the protein absorption was higher on the nonuniform surface of PCL films compared to uniform surfaces and caused greater amount of calcium deposition and higher cell proliferation.[216] Besides the size and dimension of topography, the distribution of surface topography at the surface of materials may also affect cell behavior.[217] Dalby et al. cultured hMSCs on the surface of poly(methyl methacrylate) scaffolds with different disordered nanoscale to induce the expression of bone proteins without osteogenic inducer. They suggested that controlled-disordered nanoscales (100 nm deep, 120 nm diameter, and 300 nm center–center spacing) could induce the higher expression of bone ECM components compared to other random or highly ordered nanopits.[217] Additionally, deeper nanopits influenced cell response and enhanced osteogenesis of hMSCs.[217] Connective tissue and cell basement membrane contain different collagen fibers in the micro-/nanoscale range and cells can sense and respond to specific architecture of these fibers and ECM cues in numerous ways. Therefore, creation of a micro-/nanometer topographic environment contributes to correctly mimicking ECM properties.[218] When cells are seeded on a micro-/nanoscale aligned substitute, cell orientation is adjusted along the alignment direction of the material surface.[218] One of the main characteristics of the surface topography is the direction of the dominant patterns on the surface, which refers to the surface alignment. The fiber alignment is a key factor that plays a crucial role in determining cell responses when cultured on fibrous scaffolds.[219] Wang et al. fabricated silk nanofiber hydrogels with aligned physical cues for tissue engineering purposes. They showed that the presence of aligned physical cues results in oriented morphology of cells.[220] Wismer et al. investigated the effects of oriented and nonoriented poly(ether urethane) and PCL electrospun fibers on cellular responses.[221] They observed that aligned fibers highly promote the annulus fibrosis cell phenotype with a trend toward overexpression of matrix genes compared with nonaligned fibers.[221] The higher yield strain of oriented scaffolds makes them ideal candidates for tissue engineering applications.[221] Additionally, Mi et al. reported cell migration velocity and distance between cells markedly increased on the surface of oriented scaffolds with the guidance of orientated fibers compared to scaffolds with random fiber orientation.[222] In the case of highly aligned fibers, cell elongation is highly parallel to the direction of fiber orientation, while it is random for nonorganized fibers.[223] Lins et al. selected the aligned and nonaligned fiber–based architectures to investigate their effects on the behavior of monkey NSCs (**Figure 17**A,B).[224] They found that nonaligned electrospun polyvinylidene difluoride (PVDF) fibers were suitable for both glial differentiation and NSC maintenance, whereas aligned PVDF fibers were more suitable for neuronal differentiation. In another study, MSCs seeded on the aligned fibers resulted in a tendon-like tissue formation, while on the nonaligned fibers, cells developed bone tissue at the injured region (Figure 17C).[192] They suggested that these different cellular behaviors result from cytoskeleton-mediated mechanotransduction.

Although previous studies described that modulation of the surface topography has a great influence on the cell behaviors, the mechanism remains unknown.

5.3. Surface Charge

Solid surfaces can become neutrally, positively, and negatively charged using different mechanisms such as adsorption of ions (presence of ionizable component in media), dissociation of the surface chemical groups, or application of an external electric field in aqueous solutions.[225] For example, using PLL, protamine sulfate, and poly-L-ornithine, the negatively charged

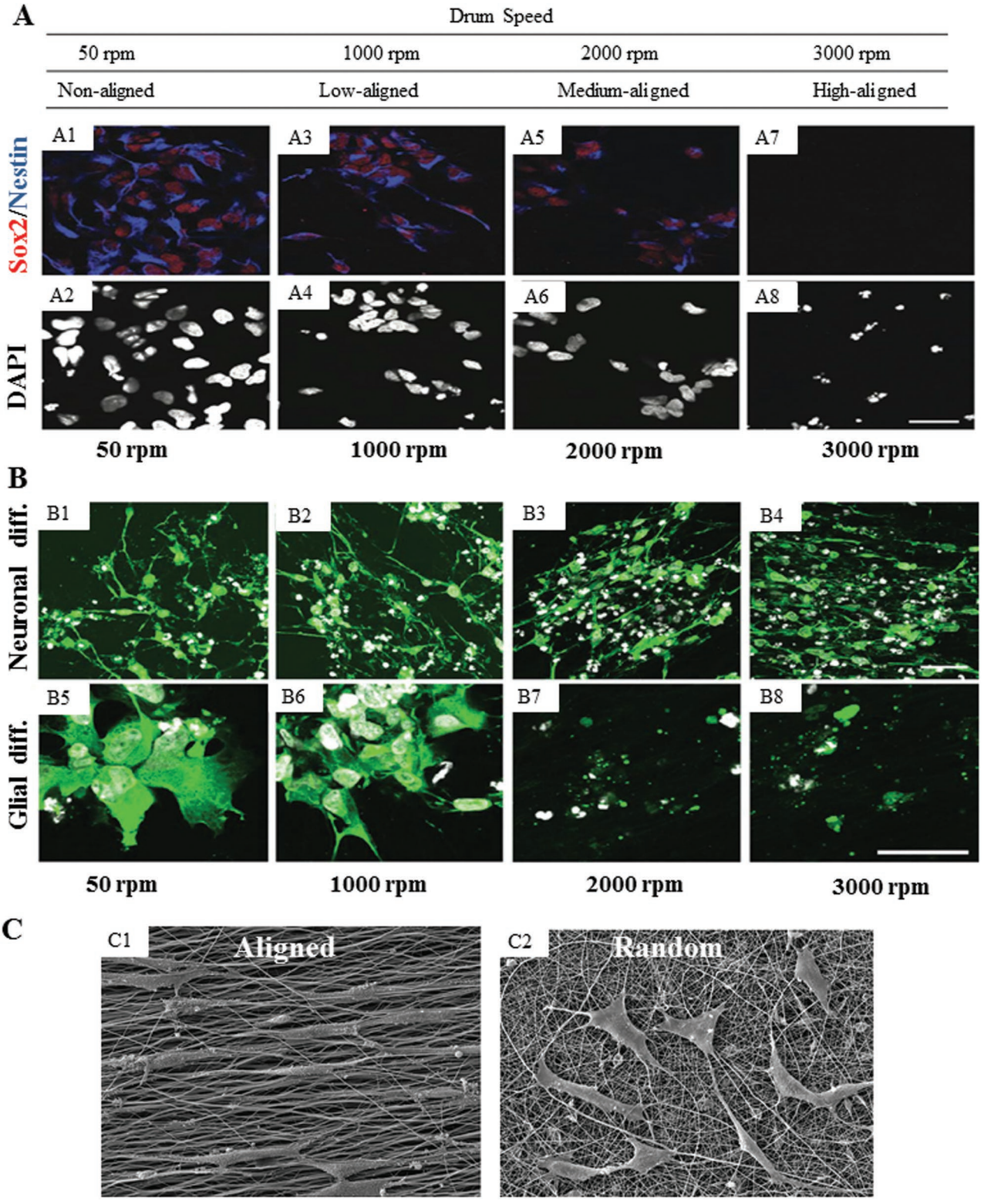

Figure 17. The effect of drum speed and fiber orientation on the cell morphology and differentiation potential. A) In comparison with aligned fibers (1000, 2000, and 3000 rpm), higher expression of Sox2/Nestin was observed in the cells cultured on nonaligned fibers (50 rpm); scale bar: 20 μm. B) Neuronal and glial differentiation of NSCs on the surface of nonaligned and aligned PVDF fibers. Scale bar: 25 μm. A,B) Reproduced with permission.[224] Copyright 2017, John Wiley & Sons. C) SEM images of multipotent MSCs cultured on the aligned (C1) and randomly oriented PLLA (C2) nanofibrous scaffolds. The MSCs were oriented in parallel with the fiber alignment, exhibited smaller size, and showed elongated morphology, whereas MSCs seeded on the randomly oriented scaffolds were spread out and displayed a polygonal phenotype. Reproduced with permission.[192] Copyright 2015, Elsevier.

surface can change into a positively charged surface. This mechanism is reversible, and after elimination of these compounds, the positive charge of surface can be returned into negative charge.[226] Moreover, more cells were attached to the positively charged surface compared to the negatively and neutrally charged surfaces.[227] The impact of surface charges on cell function has been further investigated by others.[228]

Of significance, the surface charge can also contribute in encapsulation of drugs, growth factors, or NPs into scaffolds.[229] Courtenay et al. incorporated the cationic trimethylammonium groups on the surface of cellulose scaffold by reaction with glycidyltrimethylammonium chloride and investigated the attachment of MG-63 cells.[230] They reported that a very small increase in the surface charge, without addition of any protein modifiers or ligands, markedly enhanced cell attachment onto the surface.[230] In another study, Zhu and Fang found that fibroblast attachment was much stronger on the surface of positively charged chitosan substrates compared with negatively charged *O*-carboxymethyl chitosan scaffolds.[231] This phenomenon was attributed to the electrostatic interactions between the negatively charged surface of cell membrane and cationic sites in the backbone of chitosan.[231] Furthermore, Tan et al. described the design of a positively charged hydrogel for bone tissue engineering.[232] They observed that incorporation of [2-(methacryloyloxy)ethyl]trimethylammonium chloride, a positively charged monomer, into the backbone of PEG diacrylate hydrogel led to higher protein adsorption, cell adhesion, proliferation, and differentiation. In contrast with the above-mentioned studies, Xu et al. observed higher cell number attached to the negatively charged surface of hydroxyapatite (HA) ceramics compared to positively charged surfaces.[233] In another study, Villanueva et al. functionalized the surface of iron oxide NPs using differently charged carbohydrates and investigated the internalization capability of NPs using HeLa cells.[234] Interestingly, no intracellular uptake was observed in cells incubated with neutrally charged NPs, while the positively charged NPs were efficiently internalized.[234] Asati et al. reported the same results for the positively charged aminated cerium oxide NPs using human embryonic kidney cells and cardiac myocytes.[235]

For the better understanding of material surface charges, more detailed experiments are necessary. Taken together, the current literature suggest that the surface charge can affect cell responses such as cell attachment and also dictates adsorption and subsequently internalization of NPs through cell membrane due to excellent electrostatic interactions between positively charged surface of biomaterials and negative charge of cell membrane. Finally, the effects of surface charge on the cellular responses depend on the composition of biomaterials, cell type, and tissue microenvironment.

5.4. Surface Wettability (Hydrophilicity/Hydrophobicity)

The surface wettability is the adhesive force between the liquid and solid material surface that causes the spreading of the liquid across the solid surface.[236] It is well documented that proteins tend to bind onto hydrophobic surfaces while cells are typically attached and proliferated on the hydrophilic surface.[237] It is worth noting that surface wettability can be preferentially tuned from hydrophobic to hydrophilic via manipulation of surface chemistry and surface topography.[236,238] Wei et al. examined the effect of surface wettability degree on fibroblast adhesion.[239] They used plasma polymerization and oxygen plasma treatment to incorporate O_2 functional groups onto the surface of hexamethyldisiloxane scaffolds and created several surfaces with wide range of wettability from 106° (hydrophobicity) to almost 0° (superhydrophilicity). Importantly, the hydrophilic surfaces exhibited more spreading of fibroblast cells compared to the hydrophobic surfaces. In addition, they found no significant difference in proliferation for both hydrophilic and hydrophobic surfaces. Furthermore, they reported that type of adsorbed proteins on the surface depends on the degree of surface wettability; for example, fibronectin protein dominated on the hydrophilic surfaces, whereas albumin was adsorbed on hydrophobic surfaces.[239]

Taken together, cell attachment and spreading often occur on moderately wettable surface of substrates (contact angle of 70–80°),[237–239] and the positive charge and hydrophilicity increase the cell attachment while negative charge and hydrophobicity limit the attachment.[240] Shin et al. observed the highest human BMSC (hBMSC) attachment and viability on the polyethylene (PE) scaffolds with moderately wettable surfaces (contact angles of around 57° or 65°) compared to the PE scaffolds with the hydrophilic or hydrophobic surfaces (contact angles of 48° and 97°, respectively). Moreover, hBMSCs revealed flattened and rounded morphology on the hydrophilic and hydrophobic surfaces, respectively.[241] Moreover, surface roughness and wettability are correlated and directly influence the cellular responses. The hydrophilic and rough surfaces displayed better cell adhesion and proliferation relative to hydrophobic and smooth surfaces.[242]

Previous studies investigated different materials with different surface properties, which make it difficult to draw conclusions. Overall, the surface contact angle/wettability is able to affect the cell attachment and proliferation. However, there is not much data regarding the impact of these factors on cell differentiation and function both in vitro and in vivo.

5.5. Surface Energy

Surface energy is one of the decisive factors for surface wettability of biomaterials. It is well documented that surfaces with a low surface free energy are less adhesive than those with a high surface free energy.[243] Surface free energy is defined as the increase in energy when an atom is taken up from the bulk of a material and is placed at the surface. In fact, when that surface is formed, the atoms at the newly exposed surface possess fewer nearest neighbors than same atoms in the bulk position, which is known as coordinative unsaturation of the bonds. Compared with atoms whose bonds are fully saturated in the bulk, coordinatively unsaturated atoms at the surface contain a higher energy state. The types of bonds that affect surface free energy can be classified into primary (metallic, ionic, and covalent) and secondary (van der Waals) bonds. If the dangling bonds are van der Waals bonds, the nature of surface free energy will be nonpolar, while if dangling bonds mainly are ionic and covalent

bonds, it leads to Lewis acid and base contributions to the total surface free energy.[244] In the case of smooth surfaces, contact angle measurements can be applied to evaluate the components of surface free energy. It has been reported that an ideal contact angle that provides the best surface energy to control cell proliferation and behavior is about 60–70°.[245] Surface free energy can be categorized into two groups: high-energy surfaces (comprised of materials that are covalently, ionically, or metallically bonded) such as metals and oxides with surface energies in the range of 500–5000 mN m^{-1} and low-energy surfaces (comprised of materials that are bonded by van der Waals bonds) such as molecular crystals and plastics with surface energies in the range of 5–50 mN m^{-1}.[246] Indeed, surface free energy governs water interactions, protein adsorption, and cell adhesion and affects tissue formation at the interface. Hallab et al. investigated the effects of surface energy of different biomaterials, including cobalt–chromium alloy (HS25, F-75), titanium alloy (Ti-6Al-4V), silicone rubber (SR), polytetrafluoroethylene, tantalum, and the nonmetal glass Corning tissue culture dishes, on the cellular adhesion strength.[203] They found that HS25 and 316L with total surface energies of 121.10 and 129 erg cm^{-2}, respectively, exhibited highest cellular adhesion strength (472.50 and 458.80 dyne cm^{-2}, respectively), whereas SR with total surface energy of 20.89 erg cm^{-2} showed lowest cellular adhesion strength.[203] There is a close association between surface wettability and surface energy. It has been reported that increases in the free surface energy using electrical polarization result in the improvement of surface wettability of polarized ceramic biomaterials.[247] The HA sintered in saturated water demonstrated higher polarization capacity compared with HA sintered in air, which, in turn, resulted in the increase in surface free energy and subsequently wettability. Increased surface wettability accelerated attachment of mouse osteocyte-like cells on the surface.[248] In a similar study, Lim et al. showed that the surface energy and surface wettability strongly affected cellular behaviors, cell attachment, and proliferation.[249] They examined surface energy effects on osteoblastic cell growth and mineralization on plasma-treated quartz (contact angle $\theta = 0°$) and octadecyltrichlorosilane-treated quartz ($\theta = 113°$). They concluded that hydrophilic surfaces provided possibility of homogeneous spatial osteoblastic cell growth, mineral deposition, and improvement of the quantity and quality of mineralization relative to hydrophobic surfaces.

Collectively, biomaterials with total surface energies of about 100–129 erg cm^{-2} are more suitable for tissue engineering purposes. Likewise, total surface energies of about 16–20 erg cm^{-2} are the nonoptimal range to support cell adhesion, proliferation, and differentiation.

5.6. Mechanical Properties

The stiffness of underlying substrate and local ECM are guiding the cell morphology and fate decision.[250] The importance of culturing cells on the surfaces with stiffness similar to their native tissue has been suggested in several reports.[251–254] Schuh et al. cultured chondrocytes on an elastic substrate with stiffness similar to the native chondrocyte ECM and showed that chondrocytes maintained their phenotype and expressed their specific genes. In contrast, MSCs showed polygonal morphology similar to osteoblasts when cultured on a stiffer substrate (25–40 kPa).[251] In the human body, stiffness of tissues varies from 4.25 kPa for soft tissue such as brain to 2.6 MPa for stiffer tissues such as cartilage and to 15–20 GPa for hard tissues such as bone.[252,255]

Leipzig and Shoichet studied the effect of substrate Young's elastic modulus (E_Y) (<1, 3.5, 7, 10, and 20 kPa) on the behavior of adult NSCs.[253] They showed that NSCs exhibited highest proliferation on 3.5 kPa surfaces, whereas the softest surfaces with stiffness <1 kPa were more appropriate for neuronal differentiation.[253] Moreover, softer substrates progressed axonal elongation, whereas stiffer substrates could foster formation of neuron dendrites and promote synaptogenesis.[256] Recently, several groups highlighted the impact of substrate elastic moduli on cell shape and function.[254] Kim et al. investigated the effects of compressive modulus (stiffness and softness) on cell morphology using a gradient of PVA hydrogel.[108] Cells expanded more on substrates with stiffness of ≈24 kPa, whereas a highly elongated spindle-like morphology was found with soft surfaces, ≈1 kPa (**Figure 18**A).[108] Ma et al. reported gene expression profile of NSCs onto the surface of graphene-based substrates with different elastic moduli and assessed stem cell differentiation into various cell lineages (Figure 18B).[195] Vascular smooth muscle cells (SMCs) represented contractile phenotype in materials with lower elastic modulus (within the range of 0.38–12.70 N mm^{-2} of substrate stiffness).[257] SMCs were able to organize their cytoskeleton structure on stiffer substrate and progress their spreading and polarization.[258]

These findings indicate that cells respond to the physical load in their microenvironment. The suitable stiffness of engineered material depends on the target tissue. Thus, fabrication of new kinds of substrates based on the stiffness of native tissue can be helpful, since it is well demonstrated that in addition to other surface properties, stiffness guides the cell to proliferate, grow, or die. Understanding the surface stiffness at the cell–implant interfaces is crucial in designing implants for tissue regeneration purposes. Mechanotransduction pathways are the main players in the translation of mechanical signals into chemical signals and making the cellular responses through gene expression, protein synthesis, and ultimately cellular phenotype.[259] A number of mechanosensors may also be involved in cellular mechanosensing ranging from mechanosensitive ion channels and focal adhesions in cellular membrane to cytoskeleton components such as cytoplasmic proteins, actin microtubules, and intermediate filaments, and also the cell nucleus that undergoes conformational change in response to applied load.[260] Cell nucleus has a direct role in regulation of biochemical signals that arrive in the nucleus.[259,261] Remarkable attempts have also been carried out to understand how the applied mechanical load in the cell ultimately results in cellular responses such as protein synthesis and gene expression profile.[261] It has been hypothesized that the applied forces alter protein conformation structure and result in rearrangement of cell cytoskeleton architecture and subsequently activate the cell signaling cascades that mediate mechanotransduction pathways, such as mitogen-activated protein kinase pathway. In conclusion, the

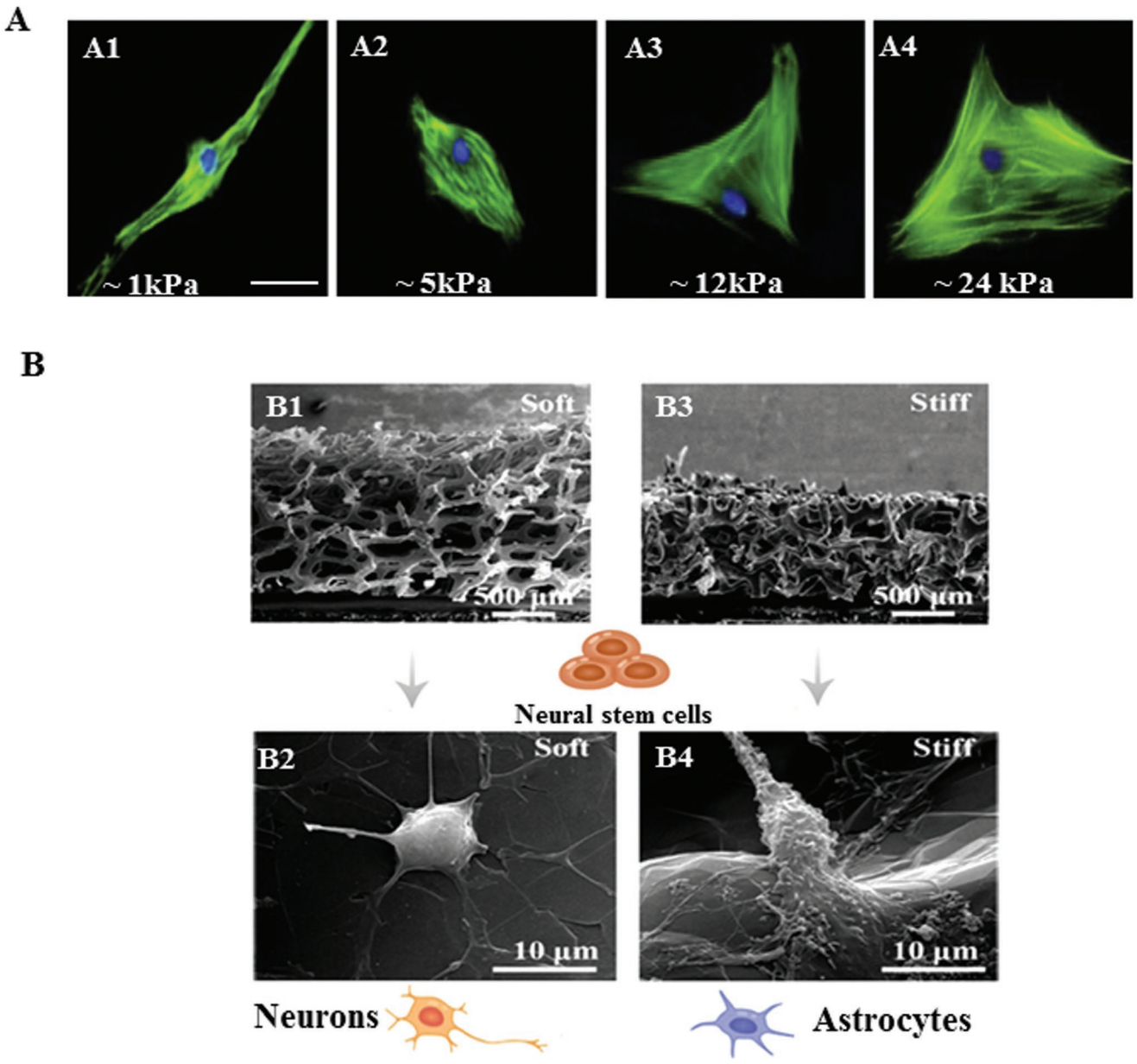

Figure 18. The effect of mechanical properties on the cell behaviors. A) Immunocytochemical staining of hBMSCs on polyvinyl alcohol hydrogel after 28 days of culture. The cell cultured on the stiff surface exhibited cell spreading, while the cells seeded on the soft surface had elongated spindle-like morphology. Reproduced with permission.[108] Copyright 2015, Elsevier. B) SEM analysis illustrates the interconnected porous structures in both soft (B1) and stiff (B3) graphene foams (GFs). The NSCs (B2) and astrocyte cells (B4) were cultured on these surfaces for 4 days. NSCs showed elongated and spreading protuberances on the surface of soft and stiff GF scaffolds, respectively. Reproduced with permission.[195] Copyright 2016, American Chemical Society.

stiffness and softness of constructs can adjust the interaction between cell and engineered implant and seem to correlate widely with cell attachment, proliferation, and morphology.

6. Conclusions and Future Perspectives

In this study, we reviewed current methods used for surface engineering of scaffolds, mainly for TERM applications. To date, many protocols have been applied to figure out the most effective approaches for immobilization of biomolecules onto the biomaterial surface and preparing the specific scaffold surfaces with customized and desired functions. High hydrophobic feature, high immunogenicity, nonspecific protein adsorption, and unfavorable physicochemical properties of most biomaterials are the main challenges of current TERM that limit the cell homing, controlled drug release in a site-specific manner, and cell attachment, proliferation, and differentiation. Application of biomaterials for TERM applications has been challenged by concerns regarding their biodegradability and cell toxicity.

Moreover, the prolonged in vivo cytotoxicity, effectiveness, and fate of surface engineered scaffolds remain unknown. Understanding the surface properties of scaffolds and their effects on cellular behavior is essential to overcome the barriers toward their design of better scaffolds. Surface modification of scaffolds by bioactive molecules for creating substrates with desired properties is a promising way to instruct cellular behavior in terms of attachment, proliferation, and differentiation. Therefore, greater insights and more thorough understanding of long-term in vivo fate of surface engineered biomaterials can expand this field toward its translation for routine clinical applications.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

cell behavior, scaffolds, surface modification, surface properties, tissue engineering

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